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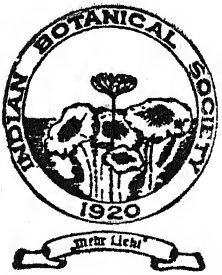
M. O. P. IYENGAR



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CORRIGENDA

Page 40, Paragraph 1, 4th line :

for Fig. 2 *read* Fig. 10

Page 40, Paragraph 2, 1st line :

for Fig. 1 *read* Fig. 9

Page 40, Paragraph 3, 9th line :

for Fig. 1 *read* Fig. 9

Plate I, facing page 42 :

for Fig. 1 *read* Fig. 9

for Fig. 2 *read* Fig. 10

Page 45, Table I, columns 4 and 5 :

for

3·654 ±0·019	0·346 ±0·027
3·877 ±0·023	0·877 ±0·032
3·960 ±0·012	0·154 ±0·010

read

3·654 ±0·027	0·346 ±0·019
3·877 ±0·082	0·877 ±0·058
3·960 ±0·036	0·487 ±0·026

Page 47, Fig. 1, about the middle :

for $\mathbf{k} = \pm 17$ *read* $\mathbf{k} = \pm 1{\cdot}1$

for $\mathbf{k} = \pm 12$ *read* $\mathbf{k} = \pm 1{\cdot}2$

Page 49, Table II, columns 3 and 4, against (*a*)—Flowering :

for ±0·110 *read* ±0·160

for ±0·160 *read* ±0·110

Plate II, facing page 64 :

for Fig. 1 *read* Fig. 2

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THE BIOLOGICAL SPECTRUM OF THE ALLAHABAD FLORA

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(Communicated by F. R. Bharucha)

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1. INTRODUCTION

IN 1905 Raunkiaer published the first comprehensive account of a Life-form System of plants which remains to-day in principle, the same. This system is simple, based mainly on *only one single feature* namely " the protection of the bud of the shoot-apices to the unfavourable season ". His main task was to describe a region in terms of the plant world. He believed that the plant climate can be characterised by a statistical survey of the life-forms.[14]

Borgesen[3] studied the vegetation of Dwarka with reference to Raunkiaer's life-forms and statistical methods, and recently Bharucha and Ferreira[12] gave an account of the Biological Spectra of Madras, Matheran, and Mahabaleshwar.

Except the work of Dudgeon[7] and the author's, *Flora of Allahabad*,[12] no work on the vegetation of this place has been done so far. Therefore, it was thought desirable to make a bio-statistical study of the flora on the basis of Raunkiaer's life-form system. This study is restricted to an area of about ten miles in radius with Allahabad as the centre. The present work on the life-forms is based not only upon the above flora, which is fairly exhaustive but help was also taken from nos. 4, 5, 6, 8, 9, 10 and 11 of the bibliography.

2. PHYSICAL FEATURES

Topography.—Allahabad is situated at 25° 26′ N. latitude and 81° 52′ E. longitude at the junction of the Ganges and the Jumna rivers. It is 319 ft. above sea-level. The area dealt with forms a part of the floristic subdivision of India, known as the Upper

Gangetic Plain. The soil is all alluvial, deposited within the recent geological times. It ranges from sand through a mixture of sand and clay, to a fine clay. The older alluvium contains deposits of $CaCO_3$ in irregular nodules called *Kankar*. Both the rivers have been depressed in the recent past, so that during the highest floods, the surrounding plains are not covered with water. Except where dissected by deep ravines, the surrounding places are almost level. Here and there are slight natural depressions which become shallow lakes, some of them drying up during summer season, others remain covered with water. On the whole the soil presents a very uniform substratum for the growth of the vegetation.

Climate.—Allahabad, as it is situated, has a strongly periodic climate characteristic of the tropical region.

Rainfall.—Both the Bay of Bengal and Arabian Sea branches of the S.W. monsoon current contribute to the rainfall of the area. The mean annual rainfall is 39·06 inches. The records of the monthly and annual rainfall are given below in Table I and the corresponding graph in Fig. 1.

From Table I it will be seen that about 94% of the rainfall occurs from June to October while only about 3% from January to February. During the monsoon the rains are at times torrential. After usually heavy rains, the level areas become vast shallow seas.

Temperature.—Table II shows the maximum, minimum and mean monthly temperatures for the year. The temperature exhibits a large range between winter and summer and between day and night, despite the fact that it is barely outside the tropics. The lowest temperature is in December 47·7° F. and then there is a rapid rise to a maximum of 106·6° F. in May.

On rare occasions in winter the temperature may go down below freezing point but these exceptional low temperatures are of little importance in determining either the character or the composition of the vegetation. The highest recorded temperature was 119·8 on June 19, 1878.

Humidity.—The mean monthly and annual humidity is given in Table III.

The lowest humidity is in the month of April and May when the temperature is maximum, and reaches its maximum 85% in August. From June onwards the percentage increases.

Wind.—During most of the year the winds blow in fits. During April, May and June, beginning from about 10 A.M. and continuing till 6 or 7 P.M., there is a strong hot wind from N.N.W. locally called the '*lu*'. Often it continues throughout the night for a couple of days in the season.

Climatic seasons.—From the foregoing it is clear that the climate is markedly periodic and can be divided into 3 seasons: (1) Rainy season, (2) Winter season and (3) Summer season.

TABLE I— *Rainfall*

Mean Rainfall	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
In inches ..	0·76	0·58	0·31	0·15	0·34	4·96	11·71	11·70	5·67	2·32	0·33	0·23	39·06
In cm. ..	1·94	1·48	0·79	0·32	0·87	12·65	29·86	29·84	14·46	5·92	0·84	0·59	99·1

TABLE II—*Temperature*

Temperature	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Maximum in ° F. ..	74·4	79·5	91·9	102·8	106·6	102·1	92·8	90·0	91·5	91·1	83·4	75·7
Minimum in ° F. ..	48·0	51·9	61·7	72·0	79·6	82·7	79·8	78·6	76·9	67·5	55·3	47·7
Mean in ° F.	61·2	65·7	76·8	87·4	93·1	92·4	86·3	84·3	83·95	78·8	69·35	61·7
Mean in ° C.	16·2	18·7	24·9	30·7	33·9	33·5	30·1	29·0	28·8	26·0	20·9	16·5

TABLE III—*Humidity*

Humidity	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Annual
Mean % ..	68	67	47	36	42	61	80	85	80	67	68	75	65·5

Corresponding with the climatic seasons, there are three distinct vegetational seasons.

Rainy season begins from about the middle of June when the first showers of rainfall and lasts till the end of September. It is characterised by high rainfall, low insolation, high temperature and high humidity. The rainy season merges gradually into the winter season which extends from begining of October to the end of February. It is characterised by low rainfall, high insolation, low temperature and relatively high humidity. This merges into the summer season extending from beginning of March to the middle of June. It is characterised by high insolation, high temperature, low humidity and strong winds.

3. The Hydrotherm Figure

The hydrotherm figure for any region, according to Raunkiaer, is a figure showing the relationship between the temperature curve, plotted in degrees Centigrade and the precipitation (rainfall) curve, plotted in centimetres in the same graph. By combining the above data of the normals of rainfall and temperature of Allahabad, the hydrotherm figure is obtained (Fig. 1). In Fig. 2, the normals of rainfall and relative humidity are plotted in the same graph.

From Tables I, II and III and Figs. 1 and 2, it is clear that the mean temperature varies between 16·2° C. in January and 33·9° C in May and June. The temperature curve shows a conspicuous trough in the months of December and January when the

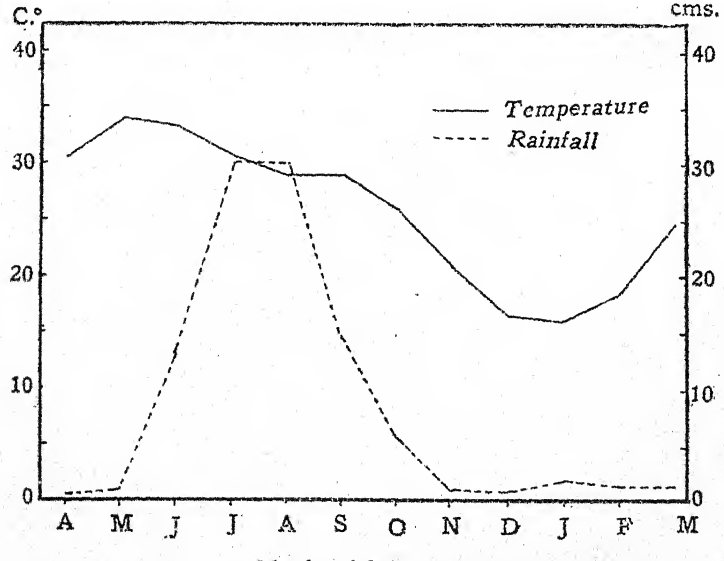

Fig. 1. Hydrotherm figure

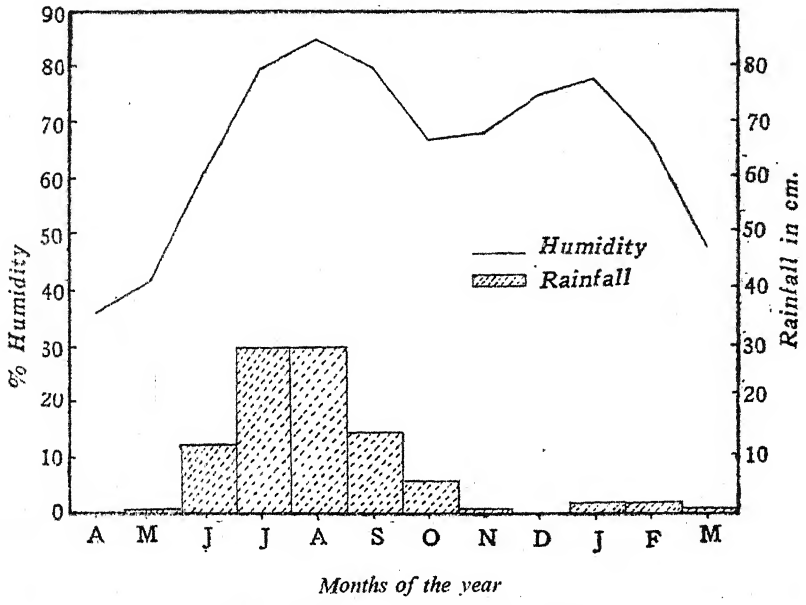

Fig. 2. Humidity and rainfall curves

temperature goes down to 16·2° C. The rainfall ranges from a little above 0 to about 29·9 cm. in July when it reaches its maximum. The precipitation curve also shows two troughs, one in November and December and the other in March–May, when the rainfall is a little above zero. As regards humidity, it is the lowest in April and the maximum in August.

The trough of the precipitation curve occurs at a different season from that of the temperature curve, so we get a dry summer and a more or less humid winter.

Thus it will be seen that the climatic factors favour the presence of two growth seasons, one during the rainy season and the other during the humid winter season, *i.e.*, January and February. The unfavourable season is from March to the middle of June when the mean rainfall is only a little above zero.

4. The Biological Spectrum

Such sub-tropical regions, with a dry summer, slightly humid winter and a pretty long rainy season in between the two seasons, are bound to be characterised by the preponderance of the annuals. In the terminology of Raunkiaer, they would be characterised as having Therophytic plant-climate. According to Raunkiaer,[13,14] Therophytes which occur in sub-tropical region survive the unfavourable season in the form of seed and complete their life-cycle within a single favourable season. That this is so is borne

out by the biological spectrum given in Table IV. Here out of the ten life-forms of terrestrial plants of Raunkiaer, I have made use of only four main types and broken up the Phanerophytes into Megaphanerophytes, Microphanerophytes, Nanophanerophytes and Lianas and grouped the rest of them, such as the succulent-stemmed, epiphytic and parasitic Phanerophytes, into one sub-group.

TABLE IV*

Regions	Number of species	The percentage distribution of the species among the life-forms								
		L	E and S.	MM.	M.	N.	Ch.	H.	Cr.	Th.
Normal ..	1,000	..	5	8	18	15	9	26	6	13
Allahabad ..	628	3·1	2·7	3	17·6	11·6	9·2	3·4	7·8	41·6
Death Valley, California	294	..	3	..	2	21	7	18	7	42

* L=Lianas, E. and S.=Epiphytes and Succulents, MM.=Megaphanerophytes, M=Microphanerophytes, N=Manophanerophytes, Ch= Chamæphytes, H=Hemicryptophytes, Cr=Cryptophytes, *i.e.* Geophytes, Helophytes, Hydrophytes, and Th=Therophytes.

In the above table are given the Biological Spectra of Allahabad, Death Valley, California, together with the Normal Spectrum. It will be seen that the life-form which exceeds most in the percentage number of plants in the spectrum for Allahabad is Therophyte 41·6% which is more than three times that of the Normal Spectrum (13%).

The groups next in importance respectively are the Microphanerophytes, the Nanophanerophytes, the Cryptophytes, and the Chamæphytes, which form 17·6%, 11·6%, 9·2% and 7·8% respectively of the total number of plants. Out of these the percentages of only the Cryptophytes and Chamæphytes exceed the corresponding figures in the Normal Spectrum, as can be seen from Table IV. Therefore the important life-forms for consideration are the Therophytes, Cryptophytes and Chamæphytes.

By applying Raunkiaer's formula, the following figures for the three life-forms are obtained :—

Thero.		Crypto.		Chamæ.
3·2	:	1·3	:	1·02

Thus the plant-climate may be characterised as Therophytic.

The percentage number of the Therophytes in Allahabad is almost the same as in the spectrum of Libyan Desert or Death Valley, California, which according to Raunkiaer, have a therophyte plant-climate. Dudgeon[7] also concluded in his study of the ecology of Allahabad that the annuals dominate the other plant

forms especially during the rainy and winter seasons and they are also present during the summer season, though the number is small.

From the above it is evident that according to Raunkiaer's terminology, Allahabad has Therophyte-plant-climate.

5. Summary and Conclusions

1. The bio-statistical study of the flora of Allahabad was made on Raunkiaer's life-form system.

2. The climate is markedly periodic and the whole year is divisible into three distinct seasons—the rainy, winter and summer seasons.

3. The biological spectrum was studied in correlation with topographical and climatic features.

4. The area shows decidedly a therophytic plant-climate with about 42% therophytes. This is confirmed by the results obtained by Dudgeon on the ecological study of this area.

Acknowledgments

In conclusion, I wish to express my deep sense of gratitude to Professor F. R. Bharucha, B.A., D.Sc., F.N.I., for his valuable suggestions and criticisms. I also thank the Director, Meteorological Observatories, Poona, for suppling me so kindly with the climatic data of Allahabad.

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VARIATION IN THE RATE OF RESPIRATION OF A GERMINATING SEED

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Received for publication on March 1, 1943

Extensive work on respiration has been done by several investigators, and many types of respirometers have been designed and recommended for estimating this activity in plants. The kinds of micro-respirometers constructed [Osterhout and Haas (1917) and Lund (1919)] and the methods recommended appear to be too complicated. The indicator method of Osterhout (1918) although efficient needs very careful handling. Davis (1925) has designed an apparatus which works on the manometer principle but the estimation of carbon dioxide is done only after 24–48 hours of respiration. The several germinating seeds likely to be introduced at a time, the possibility of accumulation of carbon dioxide in the vicinity of seeds and the long period after which the estimation is made happen to be the drawbacks in this case. A more recent contribution to this subject is by Brown (1942) who has designed an apparatus and studied the rate of gaseous exchange in the seed and cotyledons of *Cucurbita pepo*. The smallest quantity recorded by him happens to be 1/100 c.c. The duration of each one of his experiments was 48 hours during which period four estimations are made, these being at intervals of 18, 24, 42 and 48 hours from the time of starting the experiment. According to the author each estimation takes about 10 minutes, or more for greater accuracy, and necessitates certain corrections in the volume for the time lost during estimation. In all these investigations the readings are taken at long intervals and no effort seems to have been made as yet to record this activity at very short intervals. It was this which made the author to study this activity in the germinating seeds. For this work a special kind of respirometer had to be designed on the 'float and manometer' principle (Krishna Iyengar, 1942 *b*), and this in combination with the optical lever constructed by the author makes it possible to record very small volume of gases. The simple construction, high magnification, easy handling, efficient working and lastly direct observation were the points in view during the construction of the apparatus (Fig. 1). Strong caustic soda solution is used for the ready absorption of carbon dioxide evolved. Miller (1931) is of opinion that 'the use of a strong solution of an alkali in the apparatus has a disadvantage, since the ready absorption of carbon dioxide by the alkali introduces large changes in pressure within the closed apparatus thereby affecting respiration'. In the present case the bulk of respiring

material is very small, the period of investigation is short, the difference in pressure is insignificant and even then provision is made to bring up the pressure to normal. The following is an account of the apparatus and the observed variation in the rate of respiration.

Apparatus and Its Working

The trough of water (A) is meant to maintain a constant temperature in the specimen tube by directly absorbing any heat evolved during respiration. A film of oil is introduced to remove

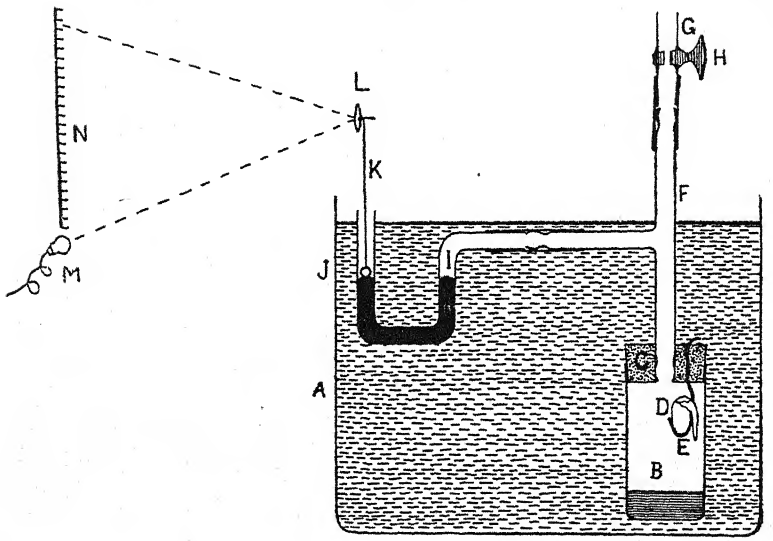

Fig. 1. Diagram of the micro-respirometer. *A*, Trough of water with a film of oil ; *B*, Specimen tube with caustic soda solution ; *C*, Rubber stopper ; *D*, Germinating seed ; *E*, Clamp ; *F*, 'T' tube; *G*, One-way glass tube ; *H*, Stop-cock ; *I*, 'U' tube with mercury ; *J*, Float ; *K*, Silk fibre ; *L*, Optical lever ; *M*, Light ; *N*, Scale.

the possibility of any fall in temperature due to evaporation of water. The tube (B) contains strong caustic soda solution at its bottom to absorb readily any carbon dioxide liberated during respiration. The germinating seed (D) is wrapped in moist blotting paper and fixed in the clamp (E). The wire from the clamp projects into the water outside. This is a device to remove immediately any heat evolved during respiration. The stopcock (H) allows or cuts off communication with the exterior, the latter being necessary before taking readings. During respiration the seed makes use of the oxygen in the chamber and liberates carbon dioxide. The ready absorption of the latter by caustic soda solution results in the rise of mercury in the closed end of the U-tube

(I). The float is attached to the short arm of the optical lever (L), the other arm being the beam of light. A galvanometric mirror mounted on the balance wheel of a watch and capable of revolving on the fine bearings forms the most important part of this optical lever. A stand with rack and pinion arrangement facilitates the proper adjustment of the optical lever and of the beam of light on the scale before recording is started. Any small depression of the float will result in the movement of the mirror in the clockwise direction, with the consequent upward movement of the focussed beam of light. Shifting the place of attachment of the float towards or away from the mirror results in a higher or lower magnification respectively. The author has made use of a mirror of 2 metre focal length, and the minimum distance at which the beam of light was focussed happens to be 2 metres. When very high magnification is necessary the distance between the scale and the mirror can be increased (this depending on the length of the room and the power of illumination) or the distance between the place of float attachment and the fulcrum reduced. By increasing the distance between the scale and the mirror to 3 metres and by reducing the distance between the fulcrum and the place of attachment to 2 mm. it is possible to have a magnification of 3,000. The movement of the beam of light projected on the scale (N) indicates the change in volume due to respiration of the seed. The shortest distance that could be observed without the help of a lens happens to be 1/40 inch. Since the diameter of the U-tube employed happens to be 4 mm. the movement of the beam of light through this distance will indicate a change in volume by nearly 1/384000 c.c., when the magnification happens to be 3,000. But for the present investigation the magnification employed was 400, thus making the smallest volume about 1/51200 c.c. for 1/40″ distance on the scale. While drawing the graphs the rates obtained from the movement of the beam at minute intervals have been doubled or quadrupled to enable a proper reproduction of the figures after reduction.

Materials and Method

The room temperature was constant during the brief period of observation in each case. The apparatus was tested before it was set up for observation. Control experiments were set up to detect the variation of pressure, if any, due to moisture. For this purpose a piece of wet blotting paper, instead of a germinating seed, was introduced into the clamp, and recording was started after an interval of an hour or more. The readings taken at minute intervals and with a very high magnification indicate that the pressure inside remains constant during a sufficiently long period. Care was taken to enable the chamber to attain saturation in humidity by introducing extra quantity of wet blotting paper for wrapping the seed. It may be stated that since the tube is a closed chamber there is every possibility of the air in the chamber reaching a stage of saturation in humidity since the quantity of

moisture lost from the blotting paper and from the seedling due to transpiration and respiration will be very much greater than the quantity of water absorbed by the surface of alkali solution during the same period. Depending on the quantity of water available in the blotting paper the stage of equilibrium in the saturation can be maintained for several hours at a stretch. Fresh solution of alkali was used for each experiment for the absorption of carbon dioxide as quickly as possible. Since the distance between the solution and the seed is very short the possibilities are in favour of small volumes of carbon dioxide settling on the solution with minimum delay, only to be readily absorbed by it.

Germinating seeds of *Dolichos lablab*, *Cicer arietinum*, *Phaseolus vulgaris*, *Pisum sativum* and *Zea Mays* were selected for studying the rate of respiration. The seed coat was carefully removed in all, except *Zea Mays*, and the germinating seed or young seedling was carefully washed and weighed before this was used for the experiments. The germinating seed was left in the apparatus for a period of about ½ hour or more before recording was started. Necessary magnification was adjusted and the readings were taken at intervals of a minute for a period of ½ to 1 hour or more. The particulars connected with the author's observations on the respiration of a germinating seed of each plant are presented in a tabular form, and the variation in the rate of respiration has been represented in the form of graphs given below.

Observations

The following tabular statements give an idea of the weight of the germinating seed, duration of experiment, magnification employed, volume of oxygen utilised, room temperature and other particulars.

The five graphs introduced in this paper show the rates of respiration in the germinating seeds of different plants. The figures 2, 3, 4, 5 and 6 are the graphs of the germinating seeds of *Dolichos lablab*, *Cicer* sp., *Phaseolus vulgaris*, *Pisum sativum* and *Zea Mays* respectively. From these figures and the data connected with each it is noticed that respiration does not go on at a uniform rate but is given to fluctuations in its rate from time to time. There are periods of high activity alternating with those of reduced ones, these resulting in the major fluctuations in the graphs. Respiration proceeds on at a high rate only for a few minutes, the period of high activity being generally between 6 to 10 minutes or more depending on the kind of young seedling. During the periods of major fluctuations there are minor fluctuations in the rate, these occurring at intervals of 1 to 2 minutes, or more. There is appreciable difference between the highest and lowest rates of this activity, the former being at times four to ten times the latter as is seen in the graphs.

Data connected with the respiration of a germinating seed of Dolichos lablab (*Fig.* 2)

Time	Movement of the beam of light (in inches) during successive minutes										Total in inches	Temperature
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th		
1st 10 minutes ..	0·80	0·80	0·70	0·65	0·50	0·65	0·70	0·55	0·65	0·60	6·60	76° F.
2nd ,, ..	0·60	0·70	0·65	0·90	0·90	0·75	0·90	0·95	..	0·75	7·20	,,
3rd ,, ..	1·05	1·00	0·75	0·85	0·60	0·85	0·75	0·80	1·00	1·10	8·75	,,
4th ,, ..	0·60	0·70	0·60	0·50	..	..	..	..	..	..	2·40	,,

Movement at the end of 33 minutes of activity .. 24·95

Weight of the germinating seed .. .. 0·812 gm.
Period of activity .. .. .. 33 minutes
Distance travelled by the beam .. .. 24·95 inches

Magnification employed .. .. ×400
Fall in the mercury column .. .. 0·156 cm.
Volume of oxygen absorbed .. .. 0·0195 c.c.

Data connected with the respiration of a germinating seed of Cicer arietinum (*Fig.* 3)

Time	Movement of the beam of light (in inches) during successive minutes										Total in inches	Temperature
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th		
1st 10 minutes ..	0·425	0·925	0·85	0·95	0·80	0·75	0·55	0·35	0·25	0·15	6·00	74·5° F.
2nd ,, ..	0·150	0·150	0·25	0·30	0·75	0·20	0·40	0·275	0·925	0·85	4·25	,,
3rd ,, ..	0·300	0·600	0·50	0·60	0·60	0·75	0·85	0·300	1·250	..	5·75	,,
4th ,, ..	..	0·400	0·10	0·20	0·30	0·25	0·15	0·200	0·200	0·15	1·95	,,
5th ,, ..	0·150	0·050	0·15	0·15	0·10	0·05	0·10	0·100	0·150	0·10	1·10	,,
6th ,, ..	0·100	0·150	0·05	0·15	0·15	0·15	0·15	0·200	0·250	0·25	1·60	,,
7th ,, ..	0·250	0·550	0·50	0·85	1·10	0·90	0·95	1·000	0·800	0·80	7·70	,,
8th ,, ..	0·900	0·900	0·85	0·90	..	..	..	..	..	..	3·55	,,

Movement at the end of 72 minutes of activity .. 31·90

Weight of the germinating seed .. .. 0·258 gm.
Period of activity .. .. .. 72 minutes
Distance travelled by the beam .. .. 31·9 inches

Magnification employed .. .. ×400
Fall in the column of mercury .. .. 0·199 cm.
Volume of oxygen absorbed .. .. 0·025 c.c.

Data connected with the respiration of a germinating seed of Phaseolus vulgaris (*Fig.* 4)

Time	Movement of the beam of light (in inches) during successive minutes										Total in inches	Temperature
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th		
1st 10 minutes ..	0·55	0·55	0·575	0·575	0·65	0·75	0·75	1·15	0·90	0·525	6·975	75° F.
2nd ,, ..	0·675	0·55	0·575	0·625	0·65	0·375	0·775	0·60	0·55	..	5·375	,,
3rd ,, ..	0·70	0·80	0·85	1·00	0·95	1·075	1·075	0·55	0·70	0·45	8·150	,,
4th ,, ..	0·55	0·50	0·325	0·25	0·325	0·650	..	..	..	..	2·600	,,

Movement at the end of 35 minutes of activity .. 23·100

Weight of the germinating seed .. .. 1·045 gm.
Period of activity .. .. .. 35 minutes
Distance travelled by the beam .. .. 23·10 inches

Magnification employed .. .. ×400
Fall in the column of mercury .. 0·144 cm.
Volume of oxygen absorbed .. .. 0·018 c.c.

Data connected with the respiration of a germinating seed of Pisum sativum (*Fig.* 5)

Time	Movement of the beam of light (in inches) during successive minutes										Total in inches	Temperature
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th		
1st 10 minutes ..	0·15	0·375	0·45	0·15	0·475	0·35	0·35	0·05	0·15	0·55	3·05	74° F.
2nd ,, ..	0·35	0·525	0·225	0·25	0·275	0·425	0·60	0·45	0·35	0·225	3·675	,,
3rd ,, ..	0·425	0·45	0·40	0·70	0·55	0·45	0·50	0·35	0·45	0·30	4·575	,,
4th ,, ..	0·50	..	..	..	..	..	..	..	..	..	0·50	,,

Movement at the end of 31 minutes of activity .. 11·80

Weight of the germinating seed .. .. 0·455 gm.
Period of activity .. .. .. 31 minutes
Distance travelled by the beam .. .. 11·80 inches

Magnification employed .. .. ×400
Fall in the column of mercury .. 0·074 cm.
Volume of oxygen absorbed .. .. 0·0093 c.c.

Data connected with the respiration of a germinating seed of Zea Mays (*Fig.* 6)

Time	Movement of the beam of light (in inches) during successive minutes										Total in inches	Temperature
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th		
1st 10 minutes ..	0·625	0·575	0·75	0·575	0·70	0·575	0·75	0·875	0·875	0·825	7·125	74° F.
2nd ,, ..	0·875	0·85	0·85	1·00	..	..	0·575	0·70	0·625	0·725	6·20	,,
3rd ,, ..	0·60	0·95	0·675	0·725	0·575	0·95	0·575	0·50	0·50	0·60	6·65	,,
4th ,, ..	0·60	0·55	0·80	..	0·40	0·425	0·25	0·675	0·65	0·40	4·75	,,
5th ,, ..	0·55	..	..	..	..	..	..	..	..	..	0·55	,,

Movement at the end of 38 minutes of activity .. 25·275

Weight of the germinating seed .. .. 0·275 gm.
Period of activity .. .. .. 38 minutes
Distance travelled by the beam .. .. 25·275 inches

Magnification employed .. .. ×400
Fall in the column of mercury .. 0·158 cm.
Volume of oxygen absorbed .. .. 0·0198 c.c.

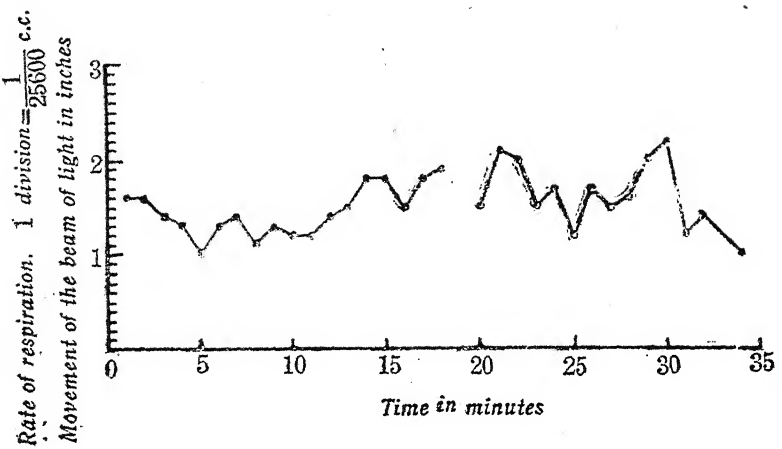

Fig. 2. Graph to show the variation in the rate of respiration in *Dolichos lablab.* ×2.

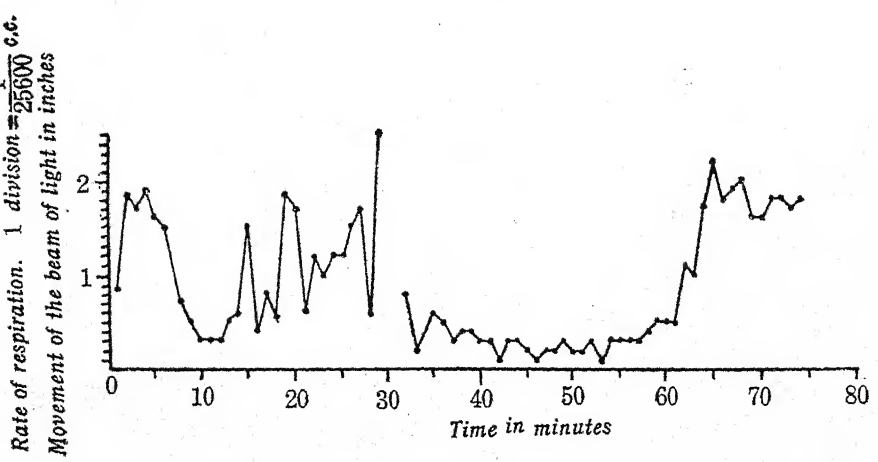

Fig. 3. Graph to show the variation in the rate of respiration in *Cicer.* ×2.

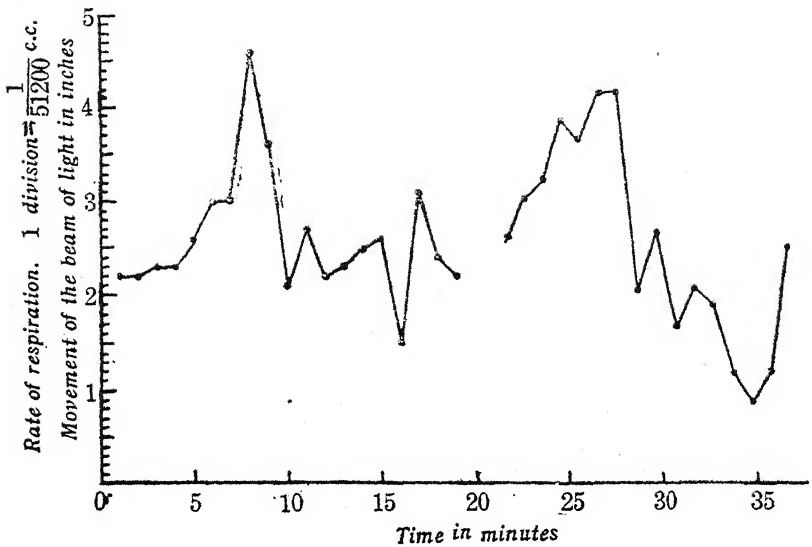

Fig. 4. Graph to show the variation in the rate of respiration in *Phaseolus vulgaris*. ×4.

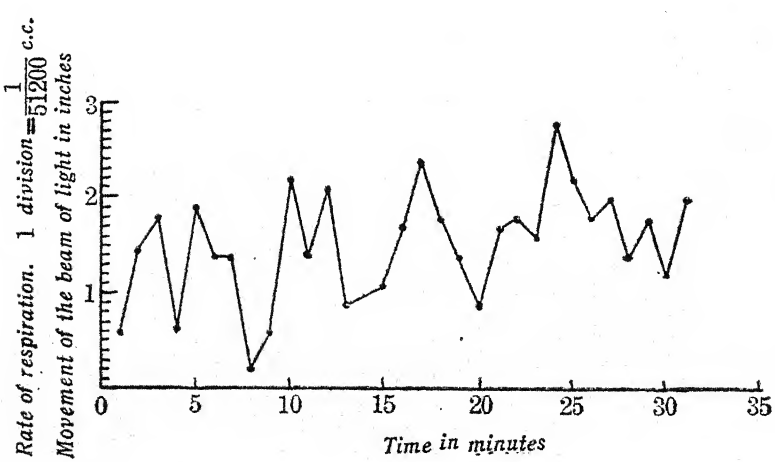

Fig. 5. Graph to show the variation in the rate of respiration in *Pisum sativum*. ×4.

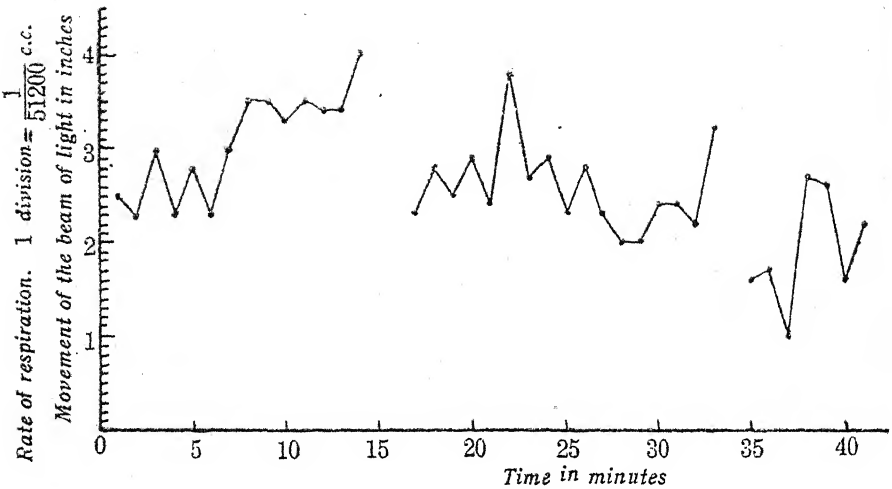

Fig. 6. Graph to show the variation in the rate of respiration in *Zea Mays*. ×4.

Discussion

From the above account it is found that notwithstanding constant external conditions there are momentary and periodical variations in the rate of respiration. A detailed enquiry into the external factors affecting respiration is out of place since the conditions are maintained almost constant during the brief periods of observation.

The experiments of Hopkins (1926) on potato illustrate not only the influence of temperature but also the effect of diastase and the accumulation of sugar on this activity. In the present case one is inclined to believe that a temporary or momentary and purely local variation in temperature within the tissues is a possibility and that this might have its own share in the variation of the activity of diastase and consequently momentary variation in the rate of respiration as shown by the minor fluctuations in the graph.

Role of moisture in respiration and its variation has been clearly explained by Bailey (1918) and other investigators. The author's observations on the leaf movements and water absorption (Krishna Iyengar, 1942 *b*) indicate that in many plants the water-content of the plant body will be varying even at short intervals of part of a minute. If similar fluctuations occur in the tissue of the germinating seed the possibilities are in favour of noticeable fluctuations in the rate of respiration also.

According to Miller (1931) the quantity of oxygen in the intercellular spaces of fruits and other plant parts primarily due

to poor gaseous exchange is often considerably below that of the air outside and may thus in some cases be limiting factor in the respiration of these parts. It is not improbable that momentary accumulation of carbon dioxide or the depletion of oxygen or both might temporarily affect respiration directly, and indirectly by affecting enzymatic activity resulting in the small oscillations at short intervals of a minute or less.

Finally the tone of the tissue may also count a great deal in deciding the rate of respiration; and its variation depends on several metabolic activities. The author's study of photosynthesis (Krishna Iyengar, 1942 *a*), leaf movement, water absorption and transpiration (Krishna Iyengar, 1942 *b*) and even growth (Krishna Iyengar, 1942 *c*) points towards the occurrence of variations in the rates of all these activities in several plants even when the external conditions are almost constant, indicating an oscillation in the rates of all these at short as well as at long intervals. Respiration shows similar fluctuations. In all these activities an active period will invariably be followed by a period of depression. These indicate the existence of possible fluctuation in the tone of the tissue from time to time, with its appreciable influence on the rates of all the vital activities. In conclusion it may be stated that while minor variations in the rate of respiration may be due to several factors like the temporary and purely local variation in the temperature due to respiration, fluctuations in the water-content, enzymatic activity and the available quantity of respirable material at a particular time and concentration of oxygen or carbon dioxide in the intercellular spaces, major variations which occur at intervals of 6 to 10 minutes or more can only be attributed to the possible fluctuations in the tone of the living tissue from time to time.

Summary

1. The germinating seeds of *Dolichos lablab*, *Cicer arietinum*, *Phaseolus vulgaris*, *Pisum sativum* and *Zea Mays* were selected for studying respiration.

2. A special micro-respirometer was designed and the readings were taken at minute-intervals and the graphs were drawn.

3. A single germinating seed was taken at a time and the rate of respiration recorded.

4. The graphs represented indicate the occurrence of major and minor fluctuations in the rate of this activity, the former generally occurring at intervals of 6 to 10 minutes or more (depending on the nature of the seed, time of the day, kind of plant, internal activities, etc.), while the latter at intervals of a minute or two—at times even less than a minute.

5. Some of the factors like temporary or momentary and purely local variations in temperature due to respiration, fluctuating water-content, enzymatic activity, available quantity

of respirable material at a particular time and variation in the concentration of carbon dioxide and oxygen in the intercellular spaces of the tissue are probably responsible for the variation at short intervals.

6. The periodic variation in the tone of the living tissue is an important factor, and this seems to be reflected in the alternating periods of high activity and depression seen not only in respiration but also in several other vital activities of the plants.

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DIFFERENTIATION OF VASCULAR TISSUES IN *HIBISCUS SABDARIFFA* LINN.

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Introduction

The importance of the fibres of *Hibiscus sabdariffa* L., particularly the variety *altissima*, as a substitute for jute has been stressed by some scientists during the last few years. The present work forms a part of the studies undertaken at the Botanical Laboratory of the Presidency College, Calcutta, on the development and structure of fibres of this plant. This paper deals with only the differentiation of the protoxylem and protophloem ; but a detailed anatomy of the plant along with the development, structure and nature of fibres is being continued and the results will be published subsequently.

Material and Methods

The specimens were obtained from plants grown in the Gardens of the Presidency College, Calcutta, from seeds received through the courtesy of the Agricultural Department of the Government of Bengal.

Tips of vegetative shoots were killed in Formalin-Acetic-Alcohol after previous treatment with Carnoy's fluid and taken through grades of alcohol and xylol in the usual way. Sections were cut 8 to 10 μ thick and stained with Safranin and Light Green or Safranin and Fast Green. The cutting of sections was often facilitated by dipping the paraffin blocks in water for 12 to 24 hours.

Observations were also made from materials macerated in 5% chromic acid ; the macerated materials were stained with Safranin or aqueous Eosin and mounted in glycerine.

Morphology

The plant is a small woody herb cultivated throughout the hotter parts of India and Ceylon. It completes its life-cycle from the germinating seed to death following fruit production within a single growing season. The stem yields a strong, silky fibre, the *Roselle hemp* of commerce, obtained by retting the stems when the plants are just in flower. It can grow in situations where jute cannot, and that is why it is believed by some that there are immense possibilities for this fibre as a substitute for jute in areas where the latter cannot be cultivated. Besides the fibre, other parts of the plant are also useful. The calyx of the flowers grows along with the fruits and becomes fleshy. It is a valuable antiscorbutic and is often eaten in the form of chutneys and jelleys. The seeds are reported to yield a kind of oil.

The plants attain a height of 8 to 10 feet and sometimes even 15 feet. The leaves are arranged spirally, the phyllotaxy varying from 2/5th in young shoots to 3/8th in vigorously growing shoots. The axillary buds produce branches which do not grow vigorously and as a result the general appearance of the plant is tall and erect.

Shoot Apex

A series of microtome sections of an actively growing stem tip across the vegetative apical bud of a vigorously growing shoot showed 8 leaves crowded around the shoot apex, which are distinctly arranged in a 3/8th phyllotaxy (Fig. 1). There is no indication of the presence of an axillary bud in the axils of the first four primordia. An axillary bud develops in the axil of the 5th primordium. The development of the first internode is initiated between the 4th and 5th primordia.

The general manner of distribution and increase in the size of the leaf primordia in the shoot apex is given below in a tabulated series :—

Primordium	Level of insertion below the shoot apex*	Depth of insertion†	Length of free limb
	μ	μ	μ
1	10	20	50
2	20	30	290
3	60	60	770
4	140	130	1890
5	240	130	..
6	460	130	..
7	660	240	..
8	1060	..	..
9	1860	..	..

* Level of insertion is the point where the leaf primordium is becoming free from the stem.

† The region between the level of insertion of the primordium and the point where it is completely fused with the axis has been termed the "depth of insertion".

Vascular Differentiation

Differentiation of vascular tissues is first noticed in the median bundle of the 3rd primordium which may be attributed to its more vigorous growth in comparison with that of primordium 2. The length of the free limb of primordium 3 was observed to be 770 μ while primordium 2 was only 290 μ in length. For this vigorous growth a greater food supply was required and as a result differentiation of vascular elements was found to take place in order to meet this demand. One protoxylem and one protophloem element was observed in this primordium (Figs. 1 and 2); the former differentiates as an isolated element at 120 μ below apex. From here the course of the xylem was traced in the free limb of this primordium and it was found to extend to a distance of 540 μ in the leaf while the phloem element continued still further up to a distance of 600 μ in this primordium. The lateral traces are also differentiated in primordium 3 but they remain in the procambial condition. The position of the protoxylem element is almost opposite to the sieve tube element of the protophloem which is also produced in the same desmogen strand.

The simultaneous differentiation of protoxylem and protophloem is contrary to the usual development of phloem before xylem, but it is not entirely unknown [see Kundu (1942) on jute].

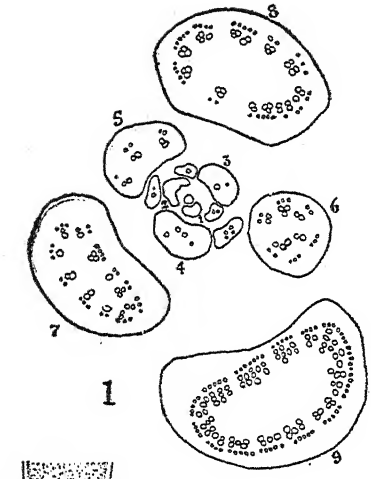
At the insertion of primordium 3 there is no distinct vacuolation of the axis, but a continuous prodesmogen strand is found to have been developed. Vacuolation of the axis commences 100 μ below the growing tip, where only a few cells in the central region are found to have been vacuolated. The process of vacuolation is very rapid and only 60 μ below this point (*i.e.*, 160 μ below the growing tip) the central region (pith) is found to have been completely vacuolated. The process of vacuolation in cortical cells also begins simultaneously with those of the central region and at the level of 180 μ below the growing tip the central as well as the cortical regions are found to have been highly vacuolated.

At the insertion of primordium 4 most of the cells in the central region are found to have been vacuolated. At the point of fusion of primordium 4 with the axis, it is found that vacuolation has started even in the ray cells giving rise to the formation of 4 strands.

The primary rays are uniseriate and the procambial cells are in more or less radial alignment resembling those of jute (Kundu, 1942).

Protoxylem

The first xylem elements to appear are the small uninucleate spiral elements. In the procambial strand 120 μ below the apex, the first indication of protoxylem vessel was noticed. A few of the cells of the procambium were found to increase markedly in length and were seen to be associated with surrounding meristematic cells, which divide radially longitudinally and irregularly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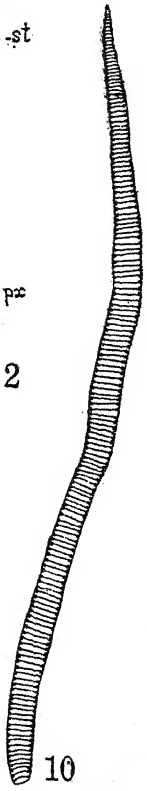
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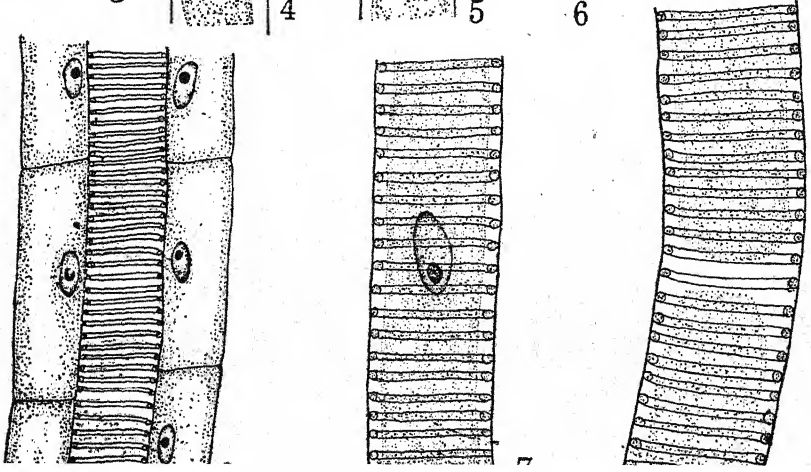
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Text-figs. 1–10.—Fig. 1. Transverse section of the apex of a vigorously growing shoot (×40). Fig. 2. Transverse section of a portion of the 3rd primordium showing differentiation of a sieve tube and a protoxylem vessel. *st.*, sieve tube; *px.*, a protoxylem vessel (×467). Fig. 3. Vessel mother cells in longitudinal section (×650). Fig. 4. File of protoplasts (×650). Fig. 5. Vessel segment with pectin film (×650). Fig. 6. Portion of a vessel segment showing deposition of bases and banding of cytoplasm. In macerated material and also in sections from fixed material the protoplasm appears to be contracted from the cell wall (×650). Fig. 7. Portion of a vessel segment with spiral secondary thickening and protoplasmic contents (×650). Fig. 8. Portion of a vessel segment with spiral thickening and degenerating cytoplasm (×650). Fig. 9. Vessel segment showing spatial adjustment of surrounding cells (×300). Fig. 10. An entire tracheid-like vessel segment at one end of which there is a perforation; the other end is pointed and has no opening (×367).

to give rise to xylem parenchyma cells. These cells are narrower than the vessel segments and are distinguished from the neighbouring pith cells by their size and protoplasmic contents. The developing vessel segments are in vertical series and they develop directly from the desmogen cells without further division.

A young vessel mother-cell is a growing meristematic cell full of contents (Fig. 3). A few such cells are arranged end to end; during the earlier stages the end walls of such cells are clearly visible separating the young vessel elements. At the first stage of vessel differentiation the transverse end walls of the vessel mother-cells are dissolved and the protoplasm is seen to contract from the transverse and longitudinal walls. Though the end walls become dissolved the protoplasts of the developing vessel segments retain their individual characteristic and they form what is called the "file of protoplasts" (Fig. 4) (Priestley, Scott and Malins, 1935). This "file of protoplasts" is formed at a very early stage in vessel differentiation when the elements are extending and expanding and its existence is for a very short period. Soon after the formation of the file of protoplasts, a film of pectin is found to be developed at the position of the end wall in the form of a membrane (Fig. 5). In a longitudinal section this "pectin film" appears to be more or less lenticular in shape and limited above and below by two dark lines enclosing a light coloured substance. It disappears at a later stage, when the vessel segment has attained its maximum extension. Sometimes it exists even when deposition of thickening matters on longitudinal walls has started. While disappearing, the pectin films first appear as faint threads; this thinning down of the pectin film indicates that it will soon be dissolved.

The first indication of secondary thickening is noticed in the deposition of bases on the inside walls of the vessel segments, when they have expanded fully (Fig. 6). These bases are thickenings on primary wall of vessel segments and they form localised projections into the cavity of vessel segments. On these projections thickening matters are laid. The presence of these projections on the wall of the vessel segment prevents the thickening matters to come into close contact with the wall. The bases appear as

continuous lines running along the centre of the thickening bands. They are not perceptible in highly lignified bands.

The protoplasm in the vessel segment suffers certain changes during the formation of the vessel. In a young cell it is uniform and dense, but as the cell increases in volume, the protoplasm contracts from the longitudinal walls as well as from the transverse end walls and fine vacuoles appear in it. Later on, with the formation of file of protoplasts these small vacuoles coalesce to form large vacuoles. In *Hibiscus* the banding of cytoplasm in finely and coarsely vacuolated regions at an early stage of vessel differentiation has not been observed as found by Barkley (1927) in *Trichosanthes* at a very high magnification. Nevertheless, when bases are deposited along the entire wall of the vessel segment the protoplast appears to be banded into thick and thin regions (Fig. 6). During all the stages of vessel differentiation and deposition of secondary thickenings the protoplast persists and the nucleus occupies central position of a vessel segment (Fig. 7). The nucleus degenerates shortly after the deposition of cellulose spiral band, but the cytoplasm persists upto the earlier stages of lignification in a fully developed vessel (Fig. 8).

It has been stated that protoplast appears to be banded after the deposition of bases. Along the thickened regions of the protoplast, deposits are formed on the walls over the bases and gradually they thicken and appear as rods. Staining reactions show that these bands are composed of pecto-cellulose but later on they change their chemical nature and become lignified.

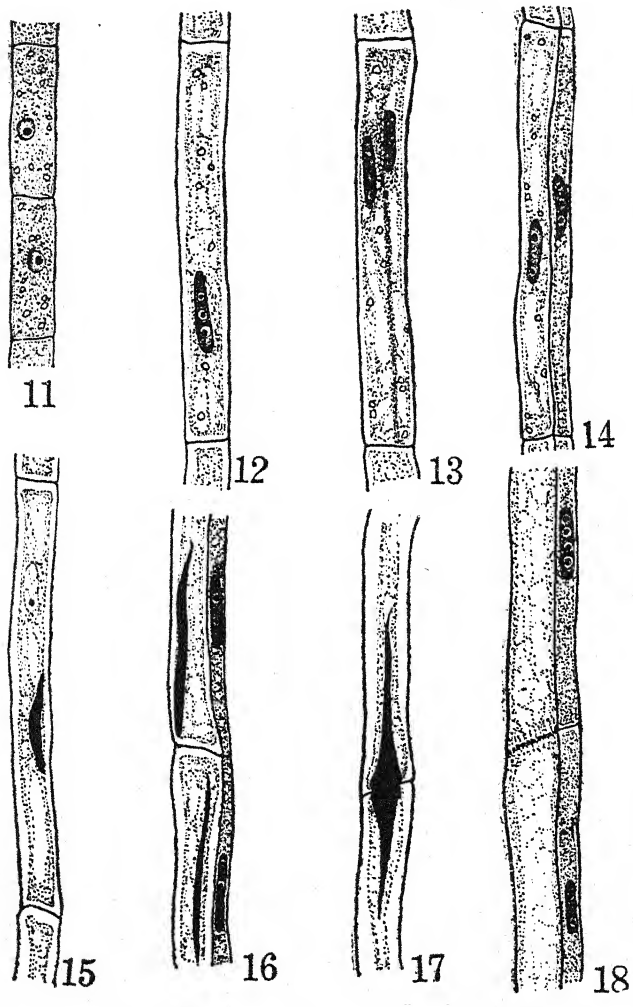
The vascular elements of the protoxylem region sometimes have pointed end walls and as a result they appear somewhat like tracheids. In Fig. 10 is shown a vessel segment at one end of which there is perforation, the other end is pointed and has no opening.

Spatial adjustment.—During the differentiation of the vessels, the vessel segments exert great pressure on the surrounding cells which are mostly meristematic. As a result, these cells undergo rapid divisions radially longitudinally, transversely or sometimes irregularly. Thus, when the vessel segment is elongating, spatial adjustment is brought about by a rapid division of the surrounding parenchyma cells (Fig. 9).

Protophloem

The ontogeny of primary sieve tubes in *Hibiscus* has been studied from transverse as well as from longitudinal sections. The development of the protophloem strand begins with the differentiation of the first sieve tube at the outer periphery of the median desmogen strand of primordium 3 (Fig. 2). In transverse sections a protophloem sieve tube can be recognised by its swollen wall which stains markedly with Light Green or Fast Green. If this element is followed downwards it is found that it soon becomes crushed and degenerated. Thus if we follow from the apex we find that a protophloem element develops from a procambial cell

and this element becomes crushed downwards due to the continued pull and pressure exerted by surrounding tissues. Hence evidently, primary sieve tubes begin to arise acropetally from those formed earlier, while the older ones are continually crushed and become obliterated.



Text-figs. 11–18.—Fig. 11. Phloem mother-cells (×975). Fig. 12. An enlarging phloem mother-cell (×975). Fig. 13. Stage showing the cutting of a companion cell (×975). Fig. 14. A young sieve tube segment with a companion cell (×975). Fig. 15. Sieve tube segment with slime body and degenerating nucleus (×975). Fig. 16. Sieve tube segments with elongated slime bodies (×975). Fig. 17. Sieve tube segments with slime plug (×975). Fig. 18. A mature sieve tube with a companion cell (×975).

The sieve tubes have one companion cell each. Phloem parenchyma consists of somewhat elongated cells with transverse walls. The companion cells are not easily recognised in transverse sections in very young regions because many of the procambium cells are similar in size to the sieve tubes and companion cells and have as dense protoplasts as the companion cells.

The development of a sieve tube from its earlier stages can best be followed from a study of longitudinal sections as well as from macerated material. A young protophloem element (a sieve tube mother-cell) is an elongated cell with heavy stained nucleus, uniform cytoplasm, and transverse end walls (Fig. 11). The nucleus contains one or more nucleoli. There are also several other small spherical particles in the mass of the protoplast and they are the characteristic sieve tube plastids.

In the next stage the sieve tube mother-cell is found to extend and the nucleus also becomes elongated and 2 or 3 nucleoli are found to be present in it. The cytoplasm also becomes less dense and vacuolated (Fig. 12).

Later on the nucleus divides with the formation of two nuclei of the same size and a wall is formed dividing the cell longitudinally into two each containing a single nucleus. One of these cells forms the sieve tube element and the other the companion cell. The young sieve tube is larger than the companion cell. The companion cells can also be differentiated from the sieve tubes by their dense protoplasts and prominent nuclei (Figs. 13 and 14).

After the companion cell has been cut off the cytoplasm of the sieve tube element becomes highly vacuolated; its nucleus loses its chromaticity, swells up and degenerates gradually. At the time the nucleus degenerates, one or more characteristic slime bodies appear in the mass of cytoplasm (Fig. 15). In the early stage they are spindle-shaped, but later on tape like slime bodies have been found in the sieve tube element (Fig. 16). The slime bodies of two adjoining sieve tube elements usually come in contact with the wall separating the two elements and form what are called the "slime plugs" (Fig. 17).

When slime plugs are formed the protoplast of the sieve tube elements contract from the longitudinal walls which become thickened. This thickening of the walls is first noticed at the stage when slime bodies appear after the cutting off of companion cell and it reaches its maximum prominence in the latest stage of development of the element. The thickened walls appear glistening and pearly in transverse sections and have been termed "nacree" walls (Leger, 1897) (Fig. 2).

After the walls have thickened, the slime plug degenerates, the end wall develops very fine perforations and the cytoplasm forms a very thin parietal layer with network of very fine strands traversing the lumen.

Discussion

Literature on the differentiation of vessels in angiosperms is scanty and the descriptions somewhat incomplete and contradictory. It is well known that the differentiation of vascular tissues is intimately connected with the development of leaves and the first differentiated vascular elements of the stem appear in localised regions which constitute the leaf traces.

In dicotyledons the differentiation of vessels usually commences in the primordium at its level of insertion on the axis and from there progresses downwards into the stem and upwards towards the apex of the primordium. This has been observed by a number of observers (Trecul, 1881, 1891; Weiss, 1883; De Bary, 1884; Priestley and Swingle, 1929; and other recent workers) and is now regarded as well established. Our observation is also in agreement with the above fact.

Regarding the relative time of appearance of the first phloem and first xylem elements, Sanio (1863) reported that in stems phloem elements appear before xylem. Russow (1872) and Leger (1897) also found the appearance of phloem before xylem; they considered it to be a normal phenomenon in the differentiation of vascular elements in Phanerogams and Cryptogams. Chang (1935) reported that the development of the vascular tissue in a primordium begins with the differentiation of a sieve tube. But in *Hibiscus* it is found that protoxylem and protophloem elements differentiate at the same time and this condition is in agreement with the observations of Kundu (1942) on jute.

According to most authors the cells destined to form the protoxylem vessels are uninucleate but F. M. Scott (1937) asserts that in *Ricinus* they are uninucleate in the younger internodes but multinucleate in the older ones. In *Hibiscus* there is no such coenocytic stage and the nucleus occupies a central or more or less central position in the vessel segment until its disintegration at the maturity of the vessel.

Regarding the breaking down of the end wall there is a great difference of opinion. According to Eames and MacDaniels (1925) the vessel segments reach their full size and permanent shape with the end walls unperforated. Esau (1936) found that in Celery the end walls break down after the maturation of the secondary wall. Barkley (1927) also refers to the late breaking down of end walls. F. M. Scott (1937) on the other hand observed that the end walls disappear in the coenocytic stage. According to Priestley and his co-workers (1935, 1938) the end walls of a vessel segment disappear at a very early stage even before the segments have attained their maximum extension. Sometimes the transverse wall splits in the middle, contracts and sticks to the sides of the vessel segments thus forming what is called the *rim*. Though the wall dissolves the protoplasts of different vessel segments do not come in contact but retain their individual characters and form what is known as "file of protoplasts". Our observations on *Hibiscus*

agree with those of Priestley, Scott and Malins (1935) and Majumdar (1940) and the "file of protoplasts" develops at a very early stage of the differentiation of the vessels. The pectin film, which becomes deposited in the position of the dissolved end wall soon after the formation of the file of protoplasts appears lenticular in shape and looks like a thickened end wall as described by Esau (1936.)

Regarding the manner of disappearance of the end walls Esau regards it as a process of dissolution. Eames and MacDaniels also describe it similarly and in *Robinia* suggested an association of the nucleus with the formation of the vessel pores. In *Hibiscus* the end walls of the developing vessel segments disappear very early, long before they attain their maximum extention. The manner of disappearance of the end walls could not be followed thoroughly. It must be an abnormally rapid process as in none of the prepared slides intermediate stages of the breaking up or the dissolution of the end walls could be traced. But it seems to us to be a process of dissolution; in no case, however, was the nucleus found to be associated with the disappearance of end walls, as its position remains unchanged and it lies at or near the centre of vessel segment until its disintegration at the maturity of the vessel. In *Hibiscus* the pectin film which is formed later and resembles the end wall described by Esau (1936), does not dissolve before the maturation of the secondary wall; sometimes, however, it disappears before the deposition of thickening matters. The nucleus does not take any part in the disappearance of the pectin film. The part played by the pectin film in the development of vessels is not quite clear.

The early stages of secondary thickening and the progress of lignification takes place while the protoplast still persists. Barkley (1927) has pointed out that before secondary thickening the protoplast is banded into alternate finely and coarsely vacuolated regions. In *Hibiscus* banding of cytoplasm has been observed simultaneously with deposition of bases. The deposition of bases before an actual secondary thickening of the vessels seems to be a special feature. This phenomenon was first noticed by Rothert (1899) (cited in Haberlandt, 1914). Eames and MacDaniels (1925), Barkley (1927), Esau (1936) and Scott (1937) did not mention anything about the deposition of bases. Recently Majumdar (1940) has observed them. The bases are formed in earlier stages and appear in the pattern of secondary thickenings on the walls. On these bases cellulose bands are deposited. The bands later on become lignified, and the protoplast of the vessel segment persists till lignification is complete.

As regards the manner of spatial adjustment during differentiation of vessels, Eames and MacDaniels state that no cells are broken during adjustment. Krabbe (1886) noticed tearing apart of adjacent cells during development of vessels and this was confirmed by Priestley, Scott and Malins (1935) in the expansion

of secondary xylem vessels. Esau (1936) has also pointed to the same method of adjustment. In *Hibiscus* spatial adjustment is brought about by active radial, longitudinal, transverse or irregular division of the surrounding cells and this agrees with the observations of Majumdar (1940) in *Heracleum*.

The failure of some workers (Eames and MacDaniels, 1925) to recognise sieve tubes in the protophloem may perhaps be attributed to their comparatively indistinct sieve plates. Nevertheless, existence of sieve tubes in the protophloem was reported by many early workers like Russow (1872) (originator of the term protophloem), De Bary (1884), Lesage (1891), Leger (1895, 1897) and Chauveaud (1897, 1900) and more recently by Chang (1935) and Esau (1934, 1935, 1936, 1938).

As regards the course of development of the protophloem, Baranetzky (1900) held that the protophloem differentiation went on simultaneously over the entire extent of the leaf trace. Griffiths and Malins (1930) have, however, observed that the protophloem elements differentiate in continuity with the phloem of the older parts of the plant, and that the first sieve tube of a particular leaf trace proceeds acropetally from the stem towards the leaf. These views have been confirmed by Chang (1935), Esau (1938) and Priestley and Scott (1938). The observations of the present writers are also in agreement with these.

The appearance of slime bodies during the development of the sieve tube has been recorded by several workers. Crafts (1933, 1934) reported that in tobacco they occur only in the primary sieve tubes. These slime bodies are generally characteristic of plants belonging to a certain family. The slime bodies in Solanaceæ have been found to be of the same nature by Kotila and Coons (1923), Doolittle and McKinney (1923), Kofoid and others (1923), Artschwager (1924), and Crafts (1933, 1934). Slime drops have been observed in the Cucurbitaceæ by Wilhelm (1880), Fischer (1886), Le Comte (1889) and Crafts (1932); and spindle-shaped slime bodies have been observed in Leguminosæ by Strasburger (1891), Baccarini (1892), Staritz (1893), Doolittle and McKinney (1923) and Bailey (1923). Some plants show no constancy of shape of the slime bodies, as in *Vitis* (Wilhelm, 1880). In *Hibiscus*, slime bodies were found to be spindle-shaped and occasionally tape-like in structure.

The nature of the slime bodies is proteinaceous and it is in agreement with the observations of several workers. Fischer (1885, 1886) reported that slime bodies become dissolved in the sieve tube sap in mature elements and according to Le Comte (1889) they disappear from the sieve tube cytoplasm by passing into the vacuole making the contents more viscous. The disappearance of slime bodies generally agrees in time with disintegration of nucleus but our observation shows that they persist even after the degeneration of the nucleus as found in the Leguminosæ by Strasburger (1890).

The majority of workers agree that the mature sieve tube does not possess any nucleus and that the sieve tube nucleus disappears as a discrete body (Wilhelm, 1880), Janczewski (1881, 1882), Schmidt (1882), Russow (1882), Strasburger (1882, 1887, 1891), Artschwager (1924), Crafts (1932, 1933, 1934), Esau (1934, 1935, 1936, 1938). Certain others report that they have seen nuclei even in the mature sieve tubes of some plants (Fischer, 1886; Le Comte, 1889; Schmidt, 1917). In *Hibiscus*, the nucleus has been found to disappear at an early stage when the slime bodies are appearing.

The sieve tube leucoplasts can be seen during the first stages of specialization of a sieve tube (Briosi, 1873; Wilhelm, 1880; Fischer, 1886; Crafts, 1934; Esau, 1934, 1935, 1936) and this corresponds with our observation on *Hibiscus*. The leucoplasts arise in the cytoplasm of young sieve tubes but may enter the vacuole in mature elements (Fischer, 1886; Crafts, 1933, 1934) and they are sometimes retained by the sieve tube until the elements begin to collapse.

Summary

1. Differentiation of protoxylem and protophloem elements takes place simultaneously in the median desmogen strand of the third leaf primordium. Vacuolation of the axial cells starts 100 μ below the growing tip and 60 μ further below it is found to be completely vacuolated.

2. The first protoxylem elements which differentiate from the desmogen are small rectangular cells (vessel mother-cells). During the first stage of vessel differentiation the longitudinal walls of the enlarging vessel mother-cells remain thin but the transverse walls disappear. The protoplasts of contiguous vessel segments do not mix but retain their individual characteristics and form "file of protoplasts". After formation of the file of protoplasts, a "pectin film" appears in the region of the transverse wall in the form of a membrane and separates the two developing vessel segments. This film which appears like an end wall is dissolved at a later stage in the development of the vessel.

3. During the process of secondary thickening of vessel segments, bases are laid down on their longitudinal walls in the pattern of the thickening matter to be deposited; on these bases cellulose bands are deposited and these bands later become lignified.

4. The protophloem element (the sieve tube mother-cell) is an elongated cell having dense protoplasm and a number of spherical granules (the sieve tube plastids). This cell rapidly extends and by its longitudinal division a companion cell is cut off. Afterwards the nucleus of the sieve tube element gradually degenerates and one or more spindle-shaped or tape-like slime bodies appear in it. At this time the longitudinal wall of the sieve tube becomes thickened and the end walls develop fine perforations. The slime bodies degenerate and the cytoplasm forms a thin

parietal layer with network of very fine strands traversing the lumen.

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MORE ABOUT *HEMILEIA CANTHII*

By S. A. Parandekar

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Received for publication on August 20, 1943

In a paper on *Hemileia Canthii* Berk. and Broome, published by M. J. Thirumalachar in the last issue of this *Journal* (Vol. XXII, Nos. 2, 3 and 4 of July 1943) it has been mentioned that this rust was collected in India on two hosts, *viz.*, *Plectronia parviflora* Bedd. and *Plectronia Rheedii* Bedd., at Yelwal, Mysore, and at Belgaum respectively.

In this connection it will be interesting to record here that the author of this note has observed the occurrence of *Hemileia Canthii* Bedd. on *Randia dumetorum* Lam. at Matheran in December 1927, and on *Plectronia Rheedii* Bedd., at Amboli Hills in January 1938, in the uredo and teleuto stages in the first case and uredo only in the second case.

For some reasons, this observation remained unpublished so far. In a recent visit (20th Dec. 1943) to Dajipore forests (Fonda ghauts), this rust was observed in the uredo stage only on the leaves of *Plectronia Rheedii* Bedd. It is not reported so far from this side.

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THE BUFFALO-HORN BAMBOO OF BURMA

An Inadequately Known Species of Giant Bamboo

BY F. G. DICKASON

Gordon College, Rawalpindi

(Communicated by Dr. R. R. Stewart, PH.D.)

Received for publication on May 31, 1943

Sinocalamus latiflorus (Munro) McClure has apparently been described only from herbarium material as the description is incomplete and taxonomically inadequate, many diagnostic characteristics not having been mentioned. Among these are some distinctive characteristics enabling field identification of the species in a vegetative condition. These are particularly important because so many species of bamboo can only be identified from the flower.

Sinocalamus latiflorus (Munro) McClure

1868 *Dendrocalamus latiflorus* Munro, *Trans. Linn. Soc.*, Vol. 26, p. 152.

1873 *Bambusa latiflora* Kurz, *Journ. As. Soc. Bengal*, Vol. 42, p. 250.

1896 *Dendrocalamus latiflorus* Munro, *Gamb. Bamb. Brit. Ind.*, p. 131.

1897 *Dendrocalamus latiflorus* Munro, Hooker, *Fl. Brit. Ind.*, Vol. 7, p. 407.

1913 *Dendrocalamus latiflorus* Munro, Camus, *Les Bambusees*, p. 160.

1921 *Dendrocalamus latiflorus* Munro, Brandis, *Ind. Trees*, p. 678.

1940 *Sinocalamus latiflorus* (Munro) McClure, in New Genera and Species of Bambusaceæ from Eastern Asia.

Material Examined

35 clumps in the field in the neighbourhood of Taunggyi in the Southern Shan States, Burma. Herbarium specimens, Judson College, Rangoon, Nos. 8288, 8372, 8678, 9397, 9398, 9399.

Description

Vegetative Characteristics.—The clumps of this species are from 80 to 100 feet high and rather open. The base of each culm leans out before turning erect, making a curve at the base of each culm like that of a water buffalo's horn (Fig. 2). The openness of the clump is accentuated by this peculiar way the culms have of leaning out before straightening up. No lateral branches are formed on new culms before April of the year following the rainy season (June to September) when the new shoots appeared. The lower 10–20 feet of the culm normally never develop lateral branches. The culms, when young, are covered with a grey-white bloom which is denser below the nodes than above. Mature stems are tough and hard, and are the ones most commonly used near Taunggyi for the posts of bamboo houses. The length of the internode $4\frac{1}{2}$ feet above the ground varies from $8\frac{1}{2}$ to 13 inches, and its circumference from $9\frac{1}{2}$ to 17 inches. The average circumference/length ratio* of the internode at $4\frac{1}{2}$ feet is $\frac{12 \cdot 25}{10 \cdot 80}$ in. or 1·14.

The culm sheath (Fig. 1) is stiff and hard, is highly polished inside, and has appressed brown hairs outside. At the lower nodes at least, the sheaths are longer than the internodes. These lower sheaths are about half again as broad as they are long, *i.e.*, 13 inches long by 22 inches broad. There are broad shoulders on the sheath, above which the sheath is truncate. There are no auricles or oral setæ. The ligule is about 5 inches broad and $\frac{1}{4}$ inch deep, the margin being serrate. The sheath blade (of a sheath at about $4\frac{1}{2}$ feet above ground) is erect and is $2\frac{1}{2}$ inches broad by $3\frac{3}{4}$ inches long. The ligule extends 1 or $1\frac{1}{4}$ inches on either side of the base of the blade which is slightly puckered before joining the sheath. There is a very distinct line separating the sheath from the blade.

Reproductive Characteristics.—When flowering occurs, the culms become great panicles with flexible, pendulous, lateral branches on which the reddish spikelets are arranged in heads at the nodes. The central lateral branch at each node is normally unbranched and on it the spikelets are in large heads of 60 or more; the internodes of this branch vary in length from 4 inches near the main culm to less than 1 inch towards its apex. All other lateral branches than the central one branch repeatedly, and on them the spikelets are arranged in smaller heads which are almost confluent (Fig. 1). These two types of inflorescence branches are so different that they might easily be ascribed to different species if seen separately.

The lemmas and palets of the florets are very pubescent and long-ciliate on margins and keels. There are no veins between the keels of the lower palets but there are as many as two between the keels of the upper ones. The apex of the anther is pointed and bristly. The long cells of the inner layer of the anther wall are peculiar in containing many annular rings of thickening.

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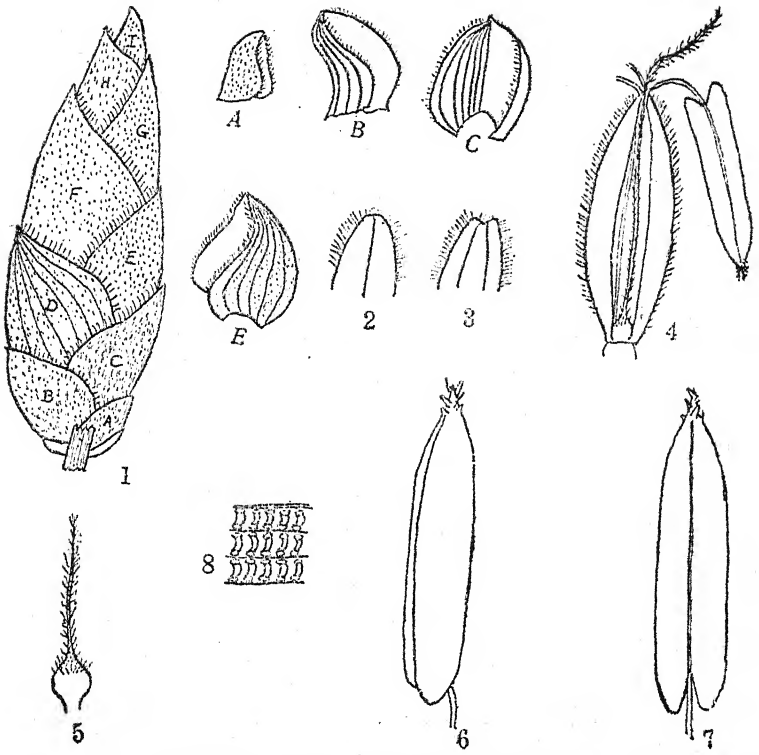
* "The Circumference-Length Ratio," *Jour. Ind. Bot. Soc.*, **21**, Nos. 5-6, 351–53



Figs. 1—8.—*Sinocalamus latiflorus* (Mun.) McClure. Fig. 1. Spikelet, 9/16 in. long, ¼ in. broad, 5/32 in. wide. A, B and C, empty glumes; D, E, F, G, H and I, lemmas of florets. Oldest floret below, youngest above. Reddish brown in colour, shortly hairy, ciliate on margins. A—Lowest empty glume, 1/8 in. long. B—Second empty glume, 3/16 in. long. C—Third empty glume, 3/16 in. long. E—Lemma of second floret, 3/8 in. long. Fig. 2. Tip of palet of E, F or G, somewhat truncate, keels long ciliate, short hairy outside, with one vein between keels. The palet of the lowest floret with acute apex and no vein between the keels. Fig. 3. Tip of palet of H or I. Very slightly bidentate, 2 veins between keels, and 1 vein in each flap. Fig. 4. Palet of D, 5/16 in. long, showing style and anther exserted. Fig. 5. Pistil: ovulary hairy on upper half, style 1, hairy. Fig. 6. Anther, yellow, side view showing bristly acuminate apex. Fig. 7. Anther, front view, 7/32 in. long. Fig. 8. Part of inner wall of anther showing peculiar annular thickening.

Records of Flowering

Sinocalamus latiflorus (Munro) McClure flowered about Taunggyi in scattered clumps during the dry seasons of 1939 to 1941, anthesis beginning during the cold season. At no place did there appear to be general flowering, although nine miles east of Taunggyi at Hopone there were half a dozen clumps flowering within a stone's throw of each other.

Vernacular Names

In the Shan villages, particularly those surrounding Taunggyi, where *Sinocalamus latiflorus* is commonly cultivated, it is called in Shan, *Mai kao quai*, which means Buffalo-horn bamboo. This vernacular name refers to the shape of the young culm shoots and to the bases of the mature culms which slope outward and then up with the same curve as that of a water buffalo's horn (Fig. 2). Other Shan names used for this bamboo are *Mai pok leng* or *Mai leng* which means Red bamboo, probably in reference to the reddish brown spikelets of the inflorescence. The same characteristic has given rise to the name *Wa ni* (Burmese) which was reported from Maymyo in 1896. The Burmese name for this species at Taunggyi is *Wa gyi*, Big bamboo ; this name, however, is used for other species of bamboo and so is not distinctive. The name, Buffalo-horn bamboo, is preferable to the names based on colour inasmuch as it refers to a distinctive vegetative characteristic which is always present.

Remarks

This species is one of the two or three kinds of giant bamboo commonly cultivated in the Shan States. The outward slant and upward curve of the culm bases of this bamboo at once set it apart in the field from the other kinds with which it commonly grows. Should a clump be found where this characteristic is not well developed, then the clear line separating the culm sheath from its blade is sufficient to distinguish this species from other large types growing in the Southern Shan States.

This Buffalo-horn bamboo may be distinguished in a number of ways from a very similar bamboo, *Mai pok mon* (Shan) (probably *Bambusa Copelandi* Gamble) which appears to be widely cultivated in the Shan States, as may be seen from the following chart :

	Mai kao quai	*Mai pok mon*
Height	.. 80–100 ft.	.. Slightly shorter
Base of culm	.. Curves out and then up	.. Lacks outward curve
Bloom on culm	.. Grey bloom denser below node than above	Grey bloom even throughout internodes
$\frac{\text{Circumference}}{\text{length}}$ ratio	.. $\frac{12.75 \text{ in.}}{11.30 \text{ in.}} = 1.13$	.. $\frac{13 \text{ in.}}{12 \text{ in.}} = 1.08.$
Uses	.. House Posts	.. Matting
Culm sheath	.. Broad shoulders and truncate across top	Curved from base rather directly to base of blade
Ligule	.. 5 inches broad	.. 3 inches broad
Culm sheath blade	.. Erect A distinct line separates blade from sheath	.. Reflexed, at least by April. No distinct line : the tissues seem continuous
Inflorescence	.. Branches drooping, pendulous	.. Branches rigid, ascending
Spikelets	.. Reddish-brown, Up to 60 or more in a head	Straw coloured, Up to 6 in a head

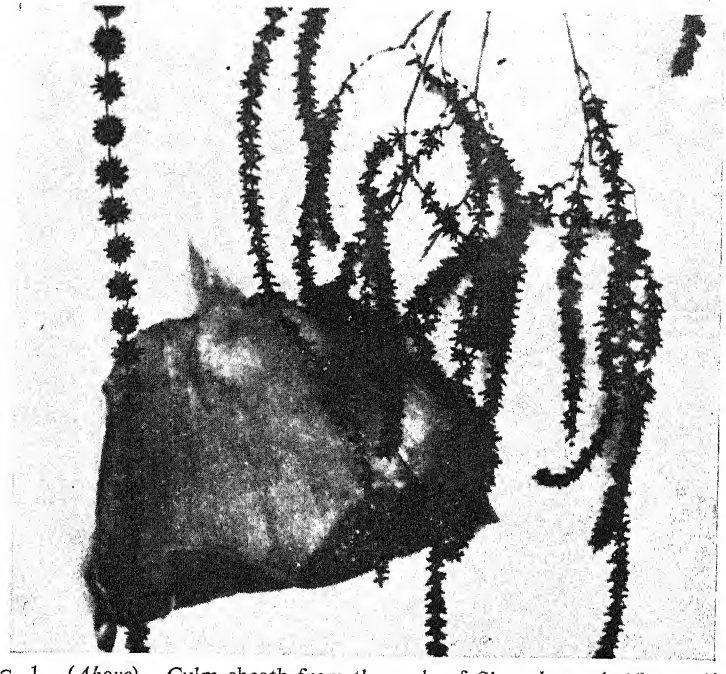


FIG. 1. (*Above*). Culm sheath from the node of *Sinocalamus latiflorus* 4½ feet above the ground, together with branches of the inflorescence, the central undivided branch being shown on the left.

FIG. 2. (*Below*). Base of a clump of *Sinocalamus latiflorus* which is in flower, showing the outward slant of the culms and the central undivided lateral branches of the inflorescence.

SUMMARY

In this paper *Sinocalamus latiflorus* (Munro) McClure of the Shan States, Burma, has for the first time been definitely linked with a Shan name, *Mai kao quai*. Additional characteristics, both vegetative and reproductive, are given which have not previously been described. A comparative chart has been worked out showing how this species differs from another similar giant bamboo, *Mai pok mon*, commonly cultivated in the same region.

VARIATION OF LEAF-FORM IN *POTAMOGETON PERFOLIATUS* L.

By R. Misra

Benares Hindu University

An opportunity to study the causes of form-variation in *Potamogeton perfoliatus* was afforded while studying the ecology of water plants in the English Lakes. Many other pond-weeds such as *P. praelongus*, *P. alpinus*, etc., also exhibit variations in the shape and size of their leaves in different lakes and even in the same lake at different places. Nevertheless, the variations in form are far more pronounced in *P. perfoliatus* than in these other species. Taxonomists usually split the natural forms into various sub-species on account of this feature (*cf.* Hagstrom, 1916). However, Fryer, Bennett and Morgan (1915) think that all the British forms of the species may possibly be mere states and not varieties. It was thought that the habitat might be partly responsible for the variations in the species and hence this study was undertaken as an aid to ecological study of the aquatic plants now published elsewhere (Misra, 1938).

P. perfoliatus is a characteristic species of silted zones in the lakes where organic matter decomposes readily. In shallower water, it grows only in land-locked bays but in deep water it can grow in exposed parts upto a depth of six metres. The plants growing in Lake Coniston have usually long internodes and narrowest leaves which are thin and olive green in colour. On the other hand, the plants of Ullswater and of the calcareous river Wharfe possess short internodes and broadest leaves which are usually thick, pale coloured and with well-developed veins. They are also usually larger in size than any other forms. The species collected from the rest of the lakes vary between these two extreme forms which differ from one another much as do shade and sun leaves. But, although the plants growing in deeper parts of a lake usually possess somewhat longer internodes yet there is apparently no correlation between depth and shape of the leaf. Thus light intensity as judged by the depth of the water has no obvious influence upon the shape of the leaf. Movement of water is also not responsible for the variations as the river forms are identical with many of the lake forms.

Shape Variations in Adult Leaves

A fully grown plant shows slightly broader leaves at the base and the top ends of the main axis than the leaves present at its middle part. The same sequence of leaf shape is found upon the individual branches although the leaves are usually much smaller in size. Since changes in the form of the leaves are mainly due to the relative variations of length and breadth the shape of the leaf can be expressed

numerically by the ratio $\frac{\text{length}}{\text{breadth}}$ (or briefly, L/B) as suggested by Pearsall and Hanby (1925). The statistics of this ratio obtained from different localities are given in Table I. The mean L/B ratios of leaves borne on different plant organs are tabulated with the standard error along with the standard deviation with its error and the variance.

TABLE I

Statistics of L/B ratios

Locality	Shoot parts	No. of leaves measured	Mean	Standard deviation	Variance
1. River Wharfe	Main axis	229	2·000 ±0·019	0·283 ±0·013	0·080
2. Lake Ullswater	Main axis	52	2·920	..	..
	Branch	35	2·810	..	..
	Flowering shoot	8	2·310	..	..
3. Lake Coniston	Main axis	156	3·776 ±0·068	0·851 ±0·048	0·724
	Branch	38	3·579 ±0·147	0·903 ±0·114	0·816
	Flowering shoot	11	2·750 ±0·091	0·301 ±0·064	0·099
4. Lake Windermere— (*a*) Low Wray Bay ..	Main axis	171	3·654 ±0·019	0·346 ±0·027	0·120
	Branch	114	3·877 ±0·023	0·877 ±0·032	0·770
(*b*) Boat house (Wray Castle)	Main axis	178	3·960 ±0·012	0·154 ±0·010	0·237
(*c*) Sawpit Bay	Main axis	115	3·210 ±0·009	..	..
	Branch	34	2·560	..	..
	Flowering shoot	22	2·410	..	..
(*d*) Greentuft Islands (Fisherty How Bay)	Main axis	112	3·200 ±0·003	0·356 ±0·002	0·127
	Flowering shoot	38	2·070	..	..
(*e*) Congo Bay	Main axis	122	3·200 ±0·050	0·553 ±0·025	0·306
(*f*) Pullwyke Bay (Deep, 3–4 metres)	Main axis	56	2·700	..	..
	Branch	8	3·500	..	..
(*g*) Pullwyke Bay (Shallow, 0·5–1 metre)	Main axis	205	2·590 ±0·020	0·285 ±0·014	0·081
	Branch	17	1·660	..	..
	Flowering shoot	14	1·820	..	..

An examination of the data solely from the point of view of the types of leaf-form shows that the leaves on the flowering branches are normally and relatively broader than those on the main axis. The other branches bear leaves which are generally of a similar L/B ratio to the main axis, though not necessarily so (*cf.* Pullwyke and Sawpit Bay samples). The production of branches in *P. perfoliatus* sometimes precedes the actual appearance of flower buds (*e.g.*, Low Wray samples) and may sometimes be more nearly associated with it in time [*e.g.*, Pullwyke (shallow) and Sawpit Bay samples]. In the former case it may be that the leaf shape resembles that of the main axis, while in the latter case it might be expected that the internal conditions would produce similar leaves on all developing branches whether flowering or not. This suggestion, however, requires further detailed examination.

Another noteworthy fact is that the maximum range of shape variation is shown by Coniston and Low Wray forms which possess the narrowest leaves. The range of shape variation on the other hand in case of broad leaved forms, *e.g.*, Wharfe and Pullwyke Bay (shallow) forms is comparatively narrow. This plasticity of the narrow leaves may be on account of a prolonged meristematic activity during which changes in growth conditions might be affecting leaf shape.

Shape Variation in Developing Leaves

A large number of young vegetative and floral buds was collected from stations at which variations in adult leaves were already studied. These buds were dissected and the young leaves measured by means of a micrometer under a microscope. The length of the developing leaves has been plotted against the breadth logarithmically for typical plant forms in Figs. 1 and 2. The curves apparently conform to the equation $x = cy^k$ as given by Pearsall (1927), where x and y are sizes of growing plant organs (corresponding to length and breadth in this study), c is a constant expressing their relative initial sizes and k is a quantity for their relative logarithmic growth rates. The value for k can be easily estimated from the slope of the curves so plotted.

Coniston forms as indicated in Fig. 1 show an initial high rate of relative growth for length with a k of the order of ± 6. However, the value for k falls down to $\pm 1 \cdot 1$ after the leaves have grown longer than 3 mm. The leaves mature so into the narrow forms. On the other hand the Wharfe type has a lower value of k (± 2) in the early phase of growth and hence develops into the broader form.

Measurements from buds taken from the top of about 6 inches high seedlings growing in Congo Bay are plotted in Fig. 2. Here the longest leaves are those first formed by the seedling ($k = \pm 1 \cdot 8$). The latter leaves show great variation but an average k of the order of ± 1. This may be held to indicate that as products of carbon assimilation become abundant k falls and this assumption would agree with the lower k for flowering plants as plotted on the same figure and as is shown subsequently for plants growing from rhizomes.

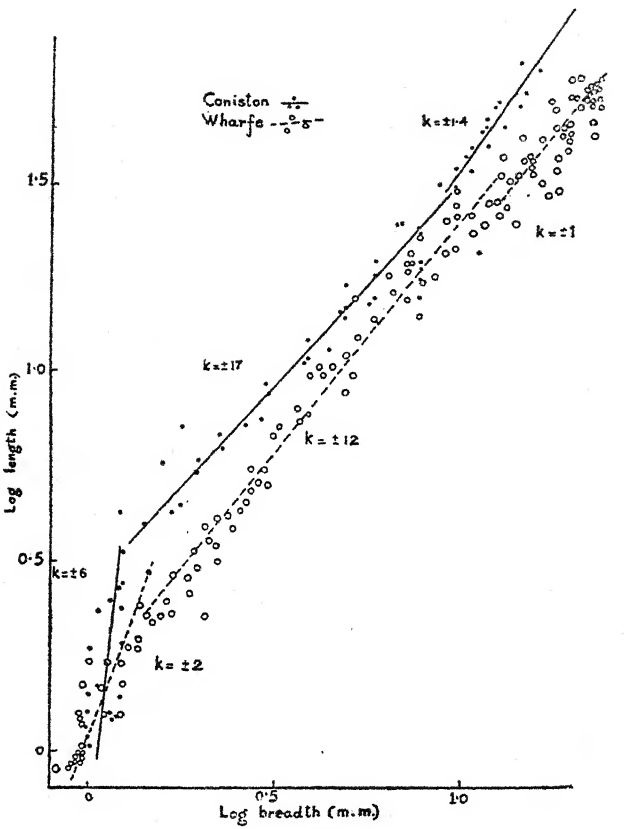


Fig. 1

Both of these in general possess broader leaves than the leaves upon a seedling or a non-flowering plant.

Two young plants apparently of the same age were found growing together from rhizome pieces of different thickness. The pieces were actually branches of the same parent rhizome. So the environmental conditions were identical for both of them. Nevertheless, the plant from the thicker rhizome had a thicker axis and the open leaves were also broader than those growing from the thinner rhizome. But on plotting logarithmic curves for the length and breadth of the young leaves from the two plants the same value for k ($\pm 1 \cdot 2$) was found for both the cases. Therefore differences in the shape of the leaves of the two plants must be on account of different values for c (in the formula $x = cy^k$) which would possibly depend upon different amount or kind of food supply from the rhizome during the stage when the leaf primordium was formed. If the food supply from the thicker rhizome is quantitatively superior in some respect to that from the narrow

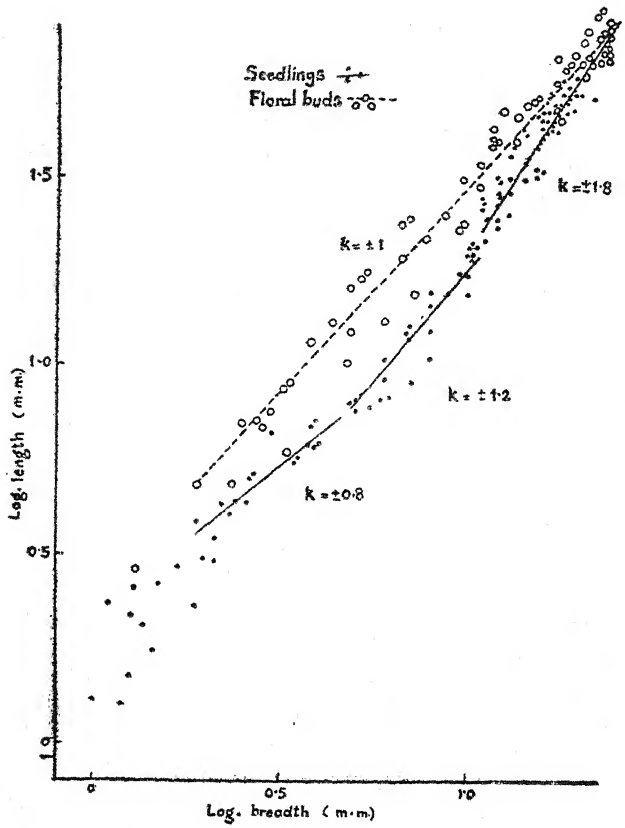


Fig. 2

one then the leaf-forms might be expected to be related to one another in much the same way as the successive leaves from a single rhizome. These become longer as they develop further from the rhizome. This is the difference observed in the examples between leaves from a narrow rhizome and those from a thick one.

As far as possible similar pieces of rhizome were obtained from Congo Bay and left in jars containing well water in the laboratory for a fortnight. The well water had a carbonate hardness two to three times more than the lake water. But when the buds developed from these rhizomes were dissected and the measurements were plotted against those developed at the same time in the lake no difference in the value of k or the shape of the leaves could be discovered. Hence it is clear that changes in the environment for a short duration cannot induce changes in the shape of the leaves which are just developing from the rhizome.

Chemical Analyses of Plants

In order to detect any obvious differences in chemical composition of the main food reserves air dried and pressed specimens were analysed for total nitrogen. The results are given in Table II. It is possible that soluble nitrogen may have been lost while pressing the plants between paper sheets and, during drying, some of the insoluble nitrogen might have been hydrolysed and redistributed in the plant. But such a preliminary study for the sake of comparison clearly showed that (*a*) buds of flowering plants have a lower nitrogen content than those from vegetative plants, (*b*) narrow leaves possess more nitrogen than broad leaves, and (*c*) the nitrogen content does not usually decrease much in older leaves of mainly vegetative plants.

Table II

Total nitrogen contents of air-dried and pressed specimens

(Expressed as percentage of dry weight)

	No. of estimations	Average	Standard deviation
(*a*) Buds—			
Vegetative	11	5·510 ±0·170	0·556 ±0·120
Flowering	7	4·550 ±0·110	0·428 ±0·160
(*b*) Narrow leaves (Coniston, Low Wray and Sawpit ; L/B = 3·2-3·76)—			
Top leaves	12	4·510 ±0·101	0·350 ±0·073
Middle leaves	5	4·330 ±0·070	0·155 ±0·049
Bottom leaves	3	3·970 ±0·120	0·203 ±0·084
(*c*) Broad leaves (Pullwyke, shallow and Ullswater ; L/B = 2·59-2·92)—			
Top leaves	7	3·810 ±0·190	0·500 ±0·130
Middle leaves	1	3·900	..
Bottom leaves	1	3·600	..

For more accurate determinations freshly collected plants of comparable age were hung on a string in a room and when sufficiently dry in air they were transferred to an oven kept at a temperature of 65° C., where these were finally dried to a constant weight. The nitrogen content of these along with their L/B ratios are shown in Table III. It will be seen here also that narrow-leaved forms are generally richer in nitrogen than the broad leaved forms.

Further analyses were made in order to obtain a comparison between the food supplies of the growing regions. In this case buds were collected and dropped into boiling alcohol and boiled for ten

TABLE III

Total nitrogen content of whole plants

(Expressed as percentage of dry weight)

Locality	L/B of leaves from axis	Total nitrogen
Coniston	3·776 ±0·068	7·567
Low Wray Bay	3·654 ±0·019	6·253
Ullswater	2·920	5·318
Pullwyke	2·590 ±0·020	4·883

minutes, then cooled and stored. Subsequently the extract and the insoluble residue were analysed separately. The results are given in Table IV. The data clearly show that vegetative buds and their leaves have a higher proportion of soluble and insoluble nitrogen than developing leaves obtained from floral buds and branches and that the ratio total sugars/soluble nitrogen is higher for young leaves

TABLE IV

Chemical analysis of buds preserved in alcohol

(Results expressed as percentage of dry weight)

Form (Locality)	Material	Dry weight, % of fresh weight	Soluble nitrogen	Insoluble nitrogen	Total sugars	Reducing sugars	Total sugars / Soluble nitrogen
Coniston ..	Vegetative buds	3·083	1·897	8·690	17·490	7·703	9·217
Ullswater ..	Young leaves from flower buds	7·082	0·805	3·368	7·674	5·835	9·528
Fisherty How Bay	Young leaves from branches	3·699	2·317	8·245	11·330	4·196	4·890
	Flower buds	3·623	0·778	5·525	7·047	4·919	8·851
	Branch buds	5·269	0·909	6·243	8·307	5·388	9·139
Congo Bay ..	Young leaves from seedlings	6·348	0·399	3·417	10·940	4·169	27·370
	Young leaves from vegetative buds	2·774	2·513	7·050	15·970	10·400	6·356
	Flower buds	6·078	0·250	2·802	3·968	2·917	15·890

obtained from floral buds or seedlings than for those obtained from vegetative buds. Thus both floral buds and seedlings seem to possess a high C/N ratio and both of them ultimately develop broad leaves. It also agrees with the general assumption that flowering is associated with higher C/N ratios.

Discussion

It has been shown that shape variations in the leaves of *Potamogeton perfoliatus* are induced when they are still developing in the bud. The most potent factor likely to bring about such changes in the bud seemed to be the quantity and quality of food supply.

It is a well-known fact that the relative rates of growing plant organs can be altered by changes in the carbon to nitrogen ratio in the growing medium. For instance, Turner (1922) and Grist and Stout (1929) have obtained a high ratio of stem/root growth by supplying a high proportion of nitrogen to the plant. The behaviour of growth in length and that in breadth of the leaves can be taken to be of similar nature since they conform to the general equation $x = cy^k$ as formulated by Pearsall (1927) and discussed at great length by Huxley (1932). In the case of *P. perfoliatus* it is found that narrower leaves are developed in localities where the substratum contains well decomposed organic matter and as has been shown by Misra (1938) it contains more of nitrogen in the available form. Thus a high supply of nitrogen from the mud and its uptake by the plant seem to increase the relative rate of growth in the length of the leaves.

The most conclusive evidence of variation in the relative rate of growth by changes in the ratio of carbon/nitrogen supply comes from an analysis of the plants. This largely happens due to changes in the metabolic balance of the growing plant organs. A high C/N ratio for instance tends in dicotyledonous plants to turn meristem into vacuolating cells. This has been shown to some extent by Pearsall and Billimoria (1938) in case of sunflower. Pearsall (unpublished) has observed in case of Sycamore that a high C/N supply to vegetative buds gives rise to narrow, small and deeply lobed leaves and longer internodes. This phenomenon is attributed to early cessation of meristematic cell divisions and increased growth of the rippen meristem. But in monocots like *P. perfoliatus* where there is a basal meristem in the leaves for quite a long period it is difficult to see how increased post-meristematic growth can alter the shape of the leaf. Nevertheless, it seems very clearly from the data presented in this work that a high C/N supply tends to produce broad leaves in *P. perfoliatus* and not narrow ones as in the case of Sycamore.

The existence of a high C/N ratio in case of *P. perfoliatus* buds is possible in three different ways. Firstly low availability of nitrogen from the mud, secondly lower reserve of nitrogen in the young leaves when the plant is flowering as has been shown to exist and thirdly by a rapid translocation of carbohydrates from the rhizome to the developing young plant from it. In all these cases the leaf tends to develop broader.

Pearsall and Hanby (1925) have shown experimentally that the leaves of *P. perfoliatus* can be made to grow broader by an increased supply of calcium in their culture medium. Hence the rooting medium and the plant ash were also analysed in this work but the results are not recorded here since no significant correlation between these data and leaf shape could be established; yet there was some indication that replaceable calcium and iron in the mud favour growth in the breadth of the leaves. Soil conditions, if they have any effect upon leaf shape which must be very complex indeed, might control growth correlations through their effect upon carbon and nitrogen metabolism of the plant.

Summary

Morphological, developmental and chemical studies of plants collected from different lakes and localities indicate that form-variation in *P. perfoliatus* is caused by an early differential growth ratio which is affected by the supply of carbohydrates to the growing organs. It has been shown that a high C/N ratio tends to decrease the differential growth ratio between length and breadth thus producing a broad leaf.

Acknowledgment

The author is indebted to Prof. W. H. Pearsall, F.R.S., for his guidance in the study.

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LIFE-HISTORY AND MORPHOLOGY OF *TROCHODIUM AJREKARI* GHARSE SP. NOV.

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Introduction

The genus *Trochodium* was founded by Sydow (1919) to accommodate the rust occurring on *Ipomœa argyreoides* Chois, in the Cape Province of South Africa and, until quite recently, contained only one species—*Trochodium Ipomœæ* (Thuem) Syd. The urediospores are stated as not formed in the description given by Dietel (1928), which is evidently an error. Doidge (1926) found a few urediospores among the teliospores in the more recent collection of the rust and it is manifest that they were present in the original specimen collected by MacOwan for Thumen who named the rust called the æcial stage *Aecidium Ipomœæ* Thumen and the uredial stage *Uredo aterrinia* Thumen, both names now included in the synonymy of the rust. That Thumen would not have given the name *Uredo aterrinia* without seeing the uredial stage goes without saying.

It may be added that Berkeley, apud Kalchbrenner (1882), found the telial stage of the rust in the same specimen which Thumen named *Uredo aterrinia* and presuming that the æcial, uredial and telial stages belonged to the same rust, he renamed it *Uromyces Ipomœæ* Berkeley, which name has been adopted by Doidge (1926), in preference to *Trochodium Ipomœæ*.

In a second species of the genus recently proposed by Thirumalachar (1942), *viz.*, *Trochodium Sampathense* on *Lettsomia elliptica* Wight and *Argyreia cymosa* Sw., pycnia, æcia and telia have been recorded ; but the rust appears to be an *opsis* form, for uredia have not been discovered and do not, presumably, occur.

A Eu-autœcious form of *Trochodium* on another member of *Convolvulaceæ*, to which family the genus seems to be restricted, is described in this paper. As far as the writer can see, the form described below is a new species which may be called *Trochodium Ajrekari* Gharse sp. nov. (?), pending its final identification. An amended description of the genus is also proposed, in view of the further discoveries of the different stages made since Sydow originally proposed it in 1919 and Dietel gave a diagnosis of it in 1928.

The writer found this *Trochodium* in October 1938 on a solitary plant of *Rivea hypocrateriformis* Choisy, on the slopes of Vetal Hill, near Poona. Though the hill abounded in plants of *Rivea hypocrateriformis*, the rust which was found in the telial stage occurred only on

a single plant. The teliospores, it was noted, germinated immediately, without a period of rest, affording a clue that the rust was probably an autœcious form.

LIFE-HISTORY

This clue was followed up by inoculation experiments on *Rivea* seedlings. Young seedlings of *Rivea* raised in pots were inoculated with germinating teliospores. On the sixth day after inoculation yellowish white specks, smaller than the head of a pin, were seen on the upper surface. These specks were found only on young tender leaves. The older leaves did not show any sign of infection. Brownish yellow pycnial dots appeared on the 8th day, singly or in groups of 2–3, in the centre of the yellow spots previously noted. Later, the number of pycnia increased, forming round pustules. The pycnia were seen on both the surfaces. Aecial swellings were first observed on the lower surface on the 6th day after the appearance of the pycnia. In some cases the æcial swellings appeared eight days after the appearance of the pycnia. In all cases, the æcia completely ruptured on the 10th day after the appearance of pycnia, or 18th day after inoculation. A very few æcia appeared on the upper surface at a very late period, when most of the lower æcia were fully ruptured.

Fresh æciospores germinated within three hours. The germinating power of these spores declined gradually till, after a fortnight, very few spores germinated.

A set of *Rivea* seedlings was inoculated on the leaves with germinating æciospores with the following result :—

Yellow spots of about ½ to 1 mm. diam. were first observed on the 9th day after the inoculation and on the 10th day small, brown, irregular eruptions could be seen, under a hand lens, which later increased in number and size and were invariably arranged in concentric circles, usually two, surrounded by a yellow halo. These were the uredia. Urediospores also germinated at once within 5–6 hours and the germination decreased as time elapsed.

Germinating urediospores were inoculated on a fresh set of *Rivea* plants in the first week of September 1940. This also produced uredia ; but these, later, were found to be intermixed with teliospores. The percentage of teliospores increased as the time passed on.

These experiments with the *Rivea* rust indicate that it is an autœcious rust and an Eu-form, a fact confirmed later, by the discovery of a few æcia in nature on the solitary plant from which the telia were originally collected.

MORPHOLOGY

The *Trochodium* on *Rivea* showed all the spore forms on the hosts in the culture experiments. Opportunity is taken, accordingly, to give an amended description of the genus. The rust on *Rivea* is proposed as a new species, *Trochodium Ajrekari* (the specific name being intended to honour my teacher, Prof. S. L. Ajrekar). It shows some important differences from the forms previously described.

Trochodium Sydow, *Ann. Mycol. Berl.*, 1919, **17**, 106

Pycnia amphigenous, subepidermal, at first in small groups, later aggregated, erumpent, flask-shaped with ostiolar paraphyses. Aecia amphigenous, but usually epiphyllous, in circles around the central group of pycnia, cupulate, with well-developed peridium ; æciospores in loose chains, polyhedral to rounded, ranging in diam. from 20 to 36 μ with one germ-pore. Uredia amphigenous, in circular patches, without paraphyses ; urediospores hyaline, echinulate, ranging in diam. from 28–38 μ, with equatorial germ-pore. Telia amphigenous, subepidermal, erumpent, scattered, minute, without paraphyses : teliospores one-celled, almost black, flattened globose, ranging in breadth from 26–33 μ and height 24–28 μ, with a flattened, fuscous papilla: epispore with regular longitudinal striæ, radiating from apex ; germ-pore apical ; pedicel hyaline, persistent, swelling in water ; germinating without a rest-period into a four-celled promycelium.

Type species.—Trochodium Ipomœæ (Thumen) Sydow, on *Ipomœæ argyreoides* Choisy, in Cape Province, South Africa

Trochodium Ajrekari Gharse, Sp. Nov.

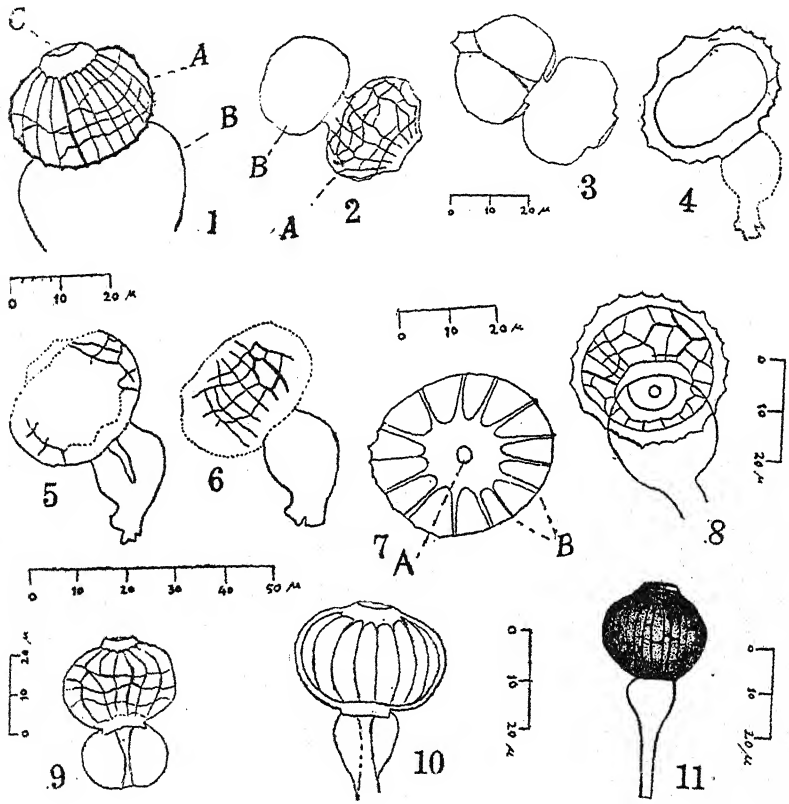
Pycnia amphigena, primo 1–3, deinde plura simul in catervas circulares coacta ; singulæ pycnium ca. 1–1·5 mm. diam., amphoræ similes, primo obscure flavæ, deinde brunneæ ; pycnia 100–140 μ diam. Ostiolum eminens, paraphysibus ornatum. Aecia epiphylla, subepidermalia, erumpentia, circulariter coacta circa, pycnia aggregata in centro, cupulata, tenuiter flava, 200–300 μ, peridio prominenti. Peridii cellulæ oblongæ vel polyhedrales, 26–33 × 19–30 μ, pariete externo dense tuberculato ; æciosporæ angulariter, globosæ, tenuiter flavæ vel hyalinæ, verrucosæ, diam. ex 22 ad 36 μ, sæpissime 26–31 μ. Uredia amphigena, diam. 3–5 mm., circumdata absque exceptione corona flava ; urediosporæ aurei coloris, 28–38 μ diam., plerumque 30–34 μ, epispora hyalina, echinulata, germinationis poro in regione equatoriali absque paraphysibus. Telia amphigena, subepidermalia, erumpentia ; teliosporæ interdum mixtæ urediosporis, obscure brunneæ, complanate globosæ, amplioris latitudinis quam longitudinis, latitudinis 26–33 μ, plerumque 28–31 μ, longitudinis 24–26 μ, raro 28 μ, ornatæ striis quæ radiorum instar emergunt ex apice et convergunt ad basim ; germinationis porus apicalis, pediculus hyalinus, persistens, longitudinis 60–65 μ, superiori parte tumescente in aqua atque cystum aliqualem efformante ; cystus 19–37 μ diam., statim germinans in 4-cellulatum promycelium.

Habitat in follis vivis *Rivea hypocrateriformis* Choisy. Typum invenit Gharse in clivo Vetal, Poona, mense Octobri 1938, atque deposuit in Herb. Crypt. Ind. Orient., in Imp. Agric. Res. Instit., New Delhi.

Trochodium Ajrekari GHARSE, SP. NOV.

Pycnia amphigenous, at first in groups of 1–3, later forming rounded groups of larger numbers, each about 1–1·5 mm. in diameter ; flask-shaped, dark yellow at first, later brown, 100–140 μ in diameter ; ostiole prominent, with ostilar paraphyses. Aecia epiphyllous, subepidermal,

erumpent, arranged in circles round the central group of pycnia, cupulate, faint yellow, 200–300 μ, with a prominent peridium ; peridial cells oblong to polyhedral, 26–33 × 19–30 μ, outer wall densely tuberculate ; æciospores angular globose, yellowish to hyaline, verrucose, ranging in diameter from 22–36 μ, mostly 26–31 μ. Uredia amphigenous, measuring 3–5 mm. in diameter, invariably surrounded by a yellow halo ; urediospores golden yellow, 28–38 μ in diameter, mostly 30–34 μ,



Figs. 1—11.—Every figure is accompanied by a scale of its magnification. The figures relate to *Trochodium* on *Rivea hypocrateriformis* unless otherwise specified.

Figs. 1 and 2. Teliospores in side view showing reticulate surface markings. (Drawn by combining different optical sectional views to bring out all the markings.) (A) Spore proper ; (B) Cyst ; (C) Germ pore. Fig. 3. The same spore as in 2 in outline (Side view). Figs. 4–6. A mature teliospore in three optical sections. Fig. 7. Top view of a teliospore showing the germ pore (A) and the longitudinal striæ (B). Fig. 8. Bottom view of a teliospore (slightly tilted) showing the attachment of the cyst and also the surface markings. Fig. 9. Markings on the surface of a mature teliospore of *Trochodium Sampathense*. (Several optical sections are combined here. A mature teliospore of *T. Ipomœæ* presents an exactly similar appearance.) Fig. 10. An immature teliospore of *T. Sampathense*. Only longitudinal markings are seen. Fig. 11. A teliospore of *T. Sampathense*. [As reproduced from Thiruma lachar (1942).]

with hyaline, echinulate epispore and a germ-pore at the equator and without paraphyses. Telia amphigenous, subepidermal, erumpent ; teliospores sometimes intermixed with urediospores, dark brown, flattened globose, broader than long, ranging in breadth from 26–33 μ, mostly 28–31 μ, height 24–26 μ, rarely 28 μ, with regular striæ radiating from the apex and converging towards the base ; germ-pore apical ; pedicel hyaline, persistent, 60–65 μ long, upper part swelling in water forming a kind of cyst ; cyst 19–37 μ in diameter ; germinating at once into a 4-celled promycelium.

Hab. on living leaves of *Rivea hypocrateriformis* Choisy, Vetal hill, Poona, Oct. 1938, Gharse (type). Type deposited in Herb. Crypt. Ind. Orient. of the Imperial Agricultural Research Institute, New Delhi.

From *Trochodium Ipomœæ*, the present species differs in having smaller æcia, but larger æciospores ; larger urediospores, and much larger telial pedicels. The cysts of *T. Ipomœæ* are moreover much smaller. *Trochodium Sampathense* differs from *Trochodium Ajrekari* in being an opsis form as proved in his culture experiments by Thirumalachar (1942) and in having smaller æciospores and teliospores.

The earlier descriptions of the markings make no reference to any lines (ridges) of latitude which in combination with longitudinal lines give a distinctly reticulate appearance to the markings with ridges enclosing alveoli or areolæ (Figs. 1, 2, 8 and 9). On closer study of the African and the South Indian specimens, however, these lines (ridges) of latitude were noticed in both, particularly in darker mature spores. In the writer's experience the colour of the mature teliospores is distinctly darker than that of immature ones and he suspects that the previous observers of *Trochodium Spp.* have failed to notice the latitudinal markings on the teliospore walls, probably because they examined only the younger spores (Fig. 10). Even in mature spores the latitudinal ridge could not be seen clearly when longitudinal ridges were focussed. Thirumalachar's (1942) fig. 6 shows some transverse ridges but the pattern is rather different from that observed by the writer (compare Fig. 1 with fig. 11 reproduced from Thirumalachar).

Summary

A species of *Trochodium* was discovered on *Rivea hypocrateriformis*, occurring on the Vetal hill near Poona. In cultural studies it was found to be an antœcious rust, showing all stages—pycnia, æcia, uredia and telia—in succession. An amended description of the genus to include all these stages is, therefore, given.

The inflated cyst at the base of the teliospore and the characteristic markings on the spore itself easily led to the identification of the form as a species of the genus *Trochodium*. A careful comparison of the African specimen—*Trochodium Ipomœæ* (Thuem) Syd., of *T. Sampathense* Thirumalachar and of *Trochodium* on *Rivea* was made. No pycnia are recorded for the African species and no uredia have been observed in connection with *T. Sampathense*. The spore-measurements

of the three forms also show sufficient differences leading to the conclusion that the *Trochodium* on *Rivea* is a new species for which the name *Trochodium Ajrekari* is proposed.

In conclusion, the writer wishes to express his indebtedness to Prof. S. L. Ajrekar, Fergusson College, Poona, for his guidance and for critically going through the manuscript and making suggestions; to Dr. B. B. Mundkur, Imperial Agricultural Research Institute, New Delhi, for help in writing the paper and supplying the literature; to Prof. V. V. Apte, Fergusson College, Poona, for giving facilities to carry on the work in the Botanical Laboratory of the Fergusson College; to Miss E. Doidge, Division of Botany, Pretoria and Mr. M. J. Thirumalachar of Central College, Bangalore, for supplying specimens of *T. Ipomœæ* and *T. Sampathense* respectively; and to Prof. Santapau of St. Xavier's College, Bombay, for the Latin rendering of the diagnosis.

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AN *ALTERNARIA* DISEASE OF SAFFLOWER*

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Introduction

Safflower (*Carthamus tinctorius* L.), an annual herbaceous plant with large orange-coloured flower heads, is grown in many parts of the world, Southern Europe, Egypt, Persia, India, China, Southern Rhodesia and South America. In India it is cultivated in Northern, Eastern, Central and Western India for its florets which are the source of a reddish dye, *carthamin*, and for its seeds from which an oil of considerable commercial importance is extracted. Safflower seed cake, a by-product during the extraction of oil, is used as a fertilizer.

In 1936 a leaf-spot disease due to a species of *Alternaria* was noticed in this crop in the Botanical Section of the Imperial Agricultural Research Institute at Pusa. It was common in the cultivators' fields at Pusa, Samastipur, Dharbhanga, Patna and Muzaffarpur wherefrom specimens were obtained and examined. It has been reported from the Central Provinces[1] but is evidently unknown in any other part of the country.

The extent of damage caused by it appears, however, to be very slight.

Symptoms of the Disease

The disease first makes its appearance just before flowering and is manifest on all parts of the plant especially the leaves.

In the beginning minute brown to dark brown spots, one to two millimetres in diameter, with concentric rings appear on the leaves. The diameter of the spots gradually increases to about one centimetre. Very often two or more adjacent spots coalesce and form large irregular lesions. The spots gradually become darker on account of the formation of the fructification. The central portion of the spot is generally light brown and is surrounded by a number of dark rings alternating with light ones. With the maturing of the spots, shot holes appear in the infected areas and if the whole leaf is attacked the blade breaks in an irregular manner due to the brittleness of the dead tissue.

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* Major portion of the work was carried out by the author in the Mycology Section of the Imperial Agricultural Research Institute.

[1] Private communication from the Mycologist, Central Provinces.

The disease is less severe on the stem and petiole where the spots are elongated. If the parasite attacks the flower buds they fail to open. Minute dark brown spots first appear at the base of the calyx ; these spots enlarge, spread and later attack other parts of the flower. The unopened flowers shrivel and dry up. Fig. 2 shows the symptoms of the disease.

Morphology of the Parasite on the Host

Mycelium.—The mycelium of the organism in the tissues of the infected area is septate and inter- and intra-cellular, with slight constrictions at the septa. The hyphæ, when young, are sub-hyaline, narrow and sparsely septate but when mature they are dark coloured, more frequently septate and broader.

Conidiophore.—The conidiophores are formed on the central dead portion of the spots. They are stout, erect, rigid, unbranched, septate and slightly constricted at the septa. They arise singly or in clusters bursting the epidermis or through the stomata, and are brown to olivaceous in colour (except the tip which is almost hyaline), about 6 to 10 μ in width, septate (the number of septa varying from 0 to 5) and rounded at the tip which is marked by a single terminal scar ; a lateral scar is sometimes also visible. The length ranges from 15 to 85 μ. Sometimes a spherical swelling (upto 12 μ in diameter) is seen at the base of the conidiophore.

Conidia.—The conidia (Fig. 1) are irregular in shape, with an apex, which is usually blunt, though tapering. Some of the smaller spores are roughly spherical and others elongate-cylindrical with rounded ends. The basal scar is usually plainly visible as also a definite

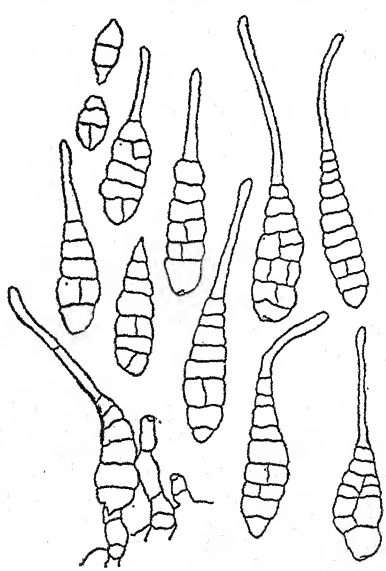

Fig. 1. Conidia of *Alternaria carthami* from nature (× 520).

apical scar, showing that a chain of at least two spores must have occurred. The conidia are light brown, translucent and a majority of them possess a long beak. They measure 36 to 171 μ with the beak, and 36 to 99μ without the beak, in length, and 12 to 28 μ in width. The conidia are usually 3 to 11-celled ; longitudinal septa are common, their number has been found to vary from 0 to 6. The beak is very lightly brown near the base and almost hyaline at the apex ; it measures 15 to 84 μ in length and 3 to 5 μ in width. A few spores without any beak sometimes occur. The surface of the conidia is smooth but sometimes with a granular appearance is surface view.

Isolation and Infection Experiments

The infected parts always showed the presence of olivaceous brown mycelium and spores of *Alternaria* species. Several single spore isolations were made and all isolations were found to be identical.

(i) *Inoculation on the Host.*—Inoculation experiments were performed on plants which had been grown from sterilized seeds. Two different methods of inoculation were followed : plants were either sprayed with spore suspension in sterile water or after spraying the plants with sterile water masses of culture containing spores and mycelium were placed on the required spots with a sterilized needle. The plants were kept covered by bell-jars for 24 or 48 hours and kept moist by occasional spraying with sterile water.

In another series, plants were inoculated by both the methods described above but they were not covered with bell-jars. The results of the inoculation experiments are summarised in Table I.

Table I

Summary of the results of inoculation experiments on Safflower by Alternaria *sp.*

Method of inoculation	Whether covered or not by bell-jars	No. of plants inoculated	No. of plants infected	No. of control plants	Controls infected
Mycelium and spores ..	Covered	72	70	32	Nil
Do. ..	,,	60	60	28	,,
Do. ..	Not covered	42	14	19	,,
Do. ..	,,	27	9	12	,,
Spore suspension ..	Covered	67	55	32	,,
Do. ..	,,	74	70	31	,,
Do. ..	Not covered	28	7	10	,,
Do. ..	,,	37	12	17	,,

It will be observed from the data presented in Table I that the presence of moisture is essential for infection ; very few plants took infection when they were not covered by bell-jars even though they were sprayed with sterile water from time to time. The method of inoculation made no difference since infection readily occurred whether

the inoculum was applied as a spore suspension or as a mass of mycelium and spores. The fungus was in every case reisolated from the infected plants. Controls were kept but in no case did they become infected.

(ii) *Cross Inoculations.*—Cross inoculation experiments were carried out on hosts which are common hosts of *Alternaria* sp. The results are recorded in Table II.

TABLE II

Cross inoculation experiments with safflower Alternaria

Hosts	No of plants inoculated	No of plants took infection
Potato (*Solanum tuberosum* L.)	27	Nil
Tomato (*Lycopersicum esculentum* Mill.) ..	17	,,
Cucumber (*Cucumis staivus* L.)	15	,,
Cotton (*Gossypium herbaceum* L.)	29	,,

Data presented in Table II show that the species of *Alternaria* isolated from safflower does not infect potato, tomato, cucumber and cotton plants.

GROWTH IN CULTURE

The fungus was grown on oat meal agar, Hopkin's agar, Dox's agar, potato-dextrose agar, Brown's standard synthetic agar and malt extract agar at room temperature (28—30° C.). The linear rate of growth was practically the same in the first four media ; it was slightly less in malt-extract agar and the least in Brown's standard synthetic agar.

In all the media in which the fungus was cultivated there was very little aerial mycelium. Mycelial growth was mostly submerged. Sometimes a tuft of floccose dark green aerial mycelium was seen around and about the inoculum. The submerged mycelium also gave the media a dark green colour. The colour around the colony was mostly white except in the potato-dextrose agar where it showed a slightly yellowish tint.

TEMPERATURE RELATIONSHIP

The linear rate of growth of the safflower *Alternaria* was studied on Hopkin's agar at various temperatures. The experiment was carried out in selected petri-dishes of uniform size into which equal amounts of the medium were poured. All the dishes were inoculated at the same time and kept at various temperatures in darkness. The experiment was run in triplicate and repeated twice. The diameters of the colonies after seven days growth are presented in Table III.

TABLE III

Growth of safflower Alternaria *at various temperatures*

Temperature (° C.)	Diameter of the colonies (mm.)
15	24
20	26
25	42
30	45
35	6

It will be observed from the data presented in Table III that the optimum temperature for growth lies between 25° and 30° C.

HYDROGEN-ION CONCENTRATION

Richards' solution as modified by Karrer and Webb (1920) was used for studying the growth rate of the fungus at different hydrogen-ion concentrations. 30 c.c. of the solution together with the required amount of N/5 acid and N/5 alkali and distilled water to make 50 c.c. was put in each flask and hydrogen-ion concentration was determined according to the colorimetric method of Clark and Lubs (1917). Four flasks were prepared for each pH value. The flasks were inoculated with a young growing culture of the fungus and incubated at room temperature (28—30° C.). After 30 days the dry weight of the mycelium was determined and is shown in Table IV.

TABLE IV

Growth of safflower Alternaria *in Richards' solution at different hydrogen-ion concentrations*

Hydrogen-ion concentration	Dry weight (mg.)
3	121
4	176
5	207
6	478
7	369
8	292
9·2	145

From the data presented in Table IV it will appear that the fungus can grow in a wide range of hydrogen-ion concentrations. The range of optimum reaction appears to lie between pH 6 and 7 and the amount of mycelium produced is the greatest at pH 6.

IDENTIFICATION OF THE PARASITE

Hitherto no species of *Alternaria* has been reported on *Carthamus tinctorius*. From a consideration of the morphology of the spore and the beak the nearest species to safflower *Alternaria* have been found

to be *Alternaria solani* (Ell. & Mart.) Jones & Grout, *A. tomato* (Cooke) Weber, *A. cucumerina* (E. & E.) Elliot and *A. macrospora* Zimm. But from the reported spore measurement data presented in Table V it will become clear that the *Alternaria* under study does not agree with any one of them.

TABLE V

Comparison of spore measurement data of safflower Alternaria *with other related species of the genus*

Fungus	Length (μ)		Total length including beak	Width (μ)
	Conidial body	Beak		
A. solani (Ell. & Mart.) Jones & Grout.	..	..	120–296	12–20
A. tomato (Cooke) Weber ..	..	..	100– 20	20–22
A. cucumerina (E. & E.) Elliot ..	30–75	25–35	55–110	15–25
A. macrospora Zimm.	..	..	150– 70	20
Safflower *Alternaria*	36–99	12–84	36–171	12–28

Some diseased leaves of safflower and a culture of the *Alternaria* sp. were sent to Dr. S. P. Wiltshire, Director, Imperial Mycological Institute, Kew, England. According to him the fungus came nearest to *A. macrospora, A. tomato, A. solani* and *A. cucumerina* but he concluded that the safflower fungus does not exactly match with any one of them.

Cross inoculation experiments carried out have shown that the *Alternaria* on safflower does not attack potato, tomato, cotton and cucumber plants. This together with the spore measurements seems to justify the establishing of this fungus as a new species for which the following name is suggested :

Alternaria carthami CHOWDHURY SP. NOV.

Alternaria carthami sp. nov.—Vegetative hyphæ septate, inter- and intra-cellular, when young sub-hyaline, narrow, sparsely septate, but when mature dark coloured, more frequently septate, broader. Conidiophores stout, erect, rigid, unbranched, septate, slightly constricted at the septa, arising singly or in clusters bursting the epidermis or through the stomata, brown to olivaceous in colour, 6 to 10 μ in width. Conidia light brown and translucent, muriform, formed at the tips of the conidiophores singly or in chains, 3 to 11-celled, longitudinal septa few, usually possessing a long beak ; conidia measure 36 to 99 μ $\times$12 to 28 μ without the beak. Beak very lightly brown near the base and almost hyaline at the apex, filiform, measuring 12 to 84 μ $\times$3 to 5 μ.

Habitat.—Parasitic on the leaves and stems of *Carthamus tinctorius* at Pusa.

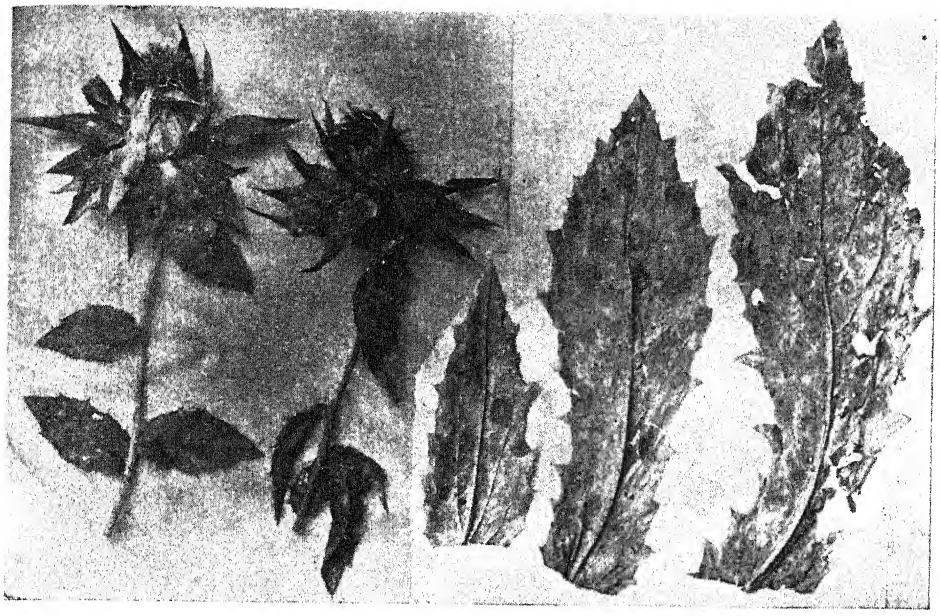

Fig. 1. Flowering twigs and leaves of *Carthamus tinctorius* showing symptoms of the disease. × 1.

Type specimen collected by S. Chowdhury in January, 1936 and deposited in the Herbarium *Cryptogamæ Indiæ Orientalis*, Imperial Agricultural Research Institute, New Delhi.

Alternaria carthami Chowdhury sp. nov.—Hyphis vegetativis septatis, inter et intra-cellularibus, primo subhyalinis, angustis, sparse septatis, aetate fuscis et sæpius septatis, latioribus. Conidiophoris crassis erectis, rigidis, simplicibus, septatis, ad septum tenuiter constrictis, singularis vel fascicularis emergentibus, ex epidermide erumpentibus vel per stomata emergentibus, brunneis, 6–10 m diam. Conidiis pallidebrunneis, pellucidis, muriformibus, ad apicibus conidiophororum singularibus vel catenulatis formantibus, 3–11 cellularis, septis longitudinalis paucis plerumque rostris longis, conidiis 36–99 ×12–28 M rostris excluderentibus. Rostro pro parte basili pallidebrunneis, pro parte apic prope hyalino filiformi, 12–84×3–5 M.

Habitat in foliis *Carthamus tinctorius* L. Pusa.

Typus in Herbarium *Cryptogamæ Indiæ Orientalis*, Imperial Agricultural Institute, New Delhi, Leg. S. Chowdhury, January, 1936.

Summary

A leaf-spot disease of *Carthamus tinctorius* was observed at Pusa and its neighbourhood. It has also been observed in other parts of Bihar and in the Central Provinces.

The disease was found to be caused by a species of *Alternaria* hitherto not reported from any part of the world. The morphology and parasitism of the fungus have been studied. It is proposed as a new species for which the name *Alternaria carthami* Chowdhury sp. nov. has been suggested.

The optimum temperature for growth has been found to lie between 25° and 30° C. and the range of optimum hydrogen-ion concentration for growth between 6 and 7.

Acknowledgments

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THE EMBRYO-SAC AND THE EMBRYO OF *SATYRIUM NEPALENSE* DON.

By B. G. L. Swamy

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The genus *Satyrium* Swartz, of the sub-tribe Diseæ belonging to the Tribe Ophrydeæ (Hooker, 1894), consists of about 50 species, which are mostly confined to Africa. *Satyrium nepalense* Don. is the only species reported from India. This plant forms a part of the characteristic vegetation of the grassy hill-top flora of the Deccan.

The plant has a subterranean unbranched tuber which commences its vegetative activity with the advent of the rainy season and the flowers are borne between July and September. The colour of the flowers varies from flake-white to deep mauve in different plants. The material for the present investigation was collected from Kodaikanal and Bababudan regions.

Megasporogenesis

The origin of the female archesporial initials in the ovary can be recognised while the pollinia of the same flower show the mature 2-nucleate condition which is the shedding stage. The archesporial cell differentiates sub-epidermally and becomes conspicuous with rich cell contents. This functions directly as the megaspore mother cell (Fig. 1).

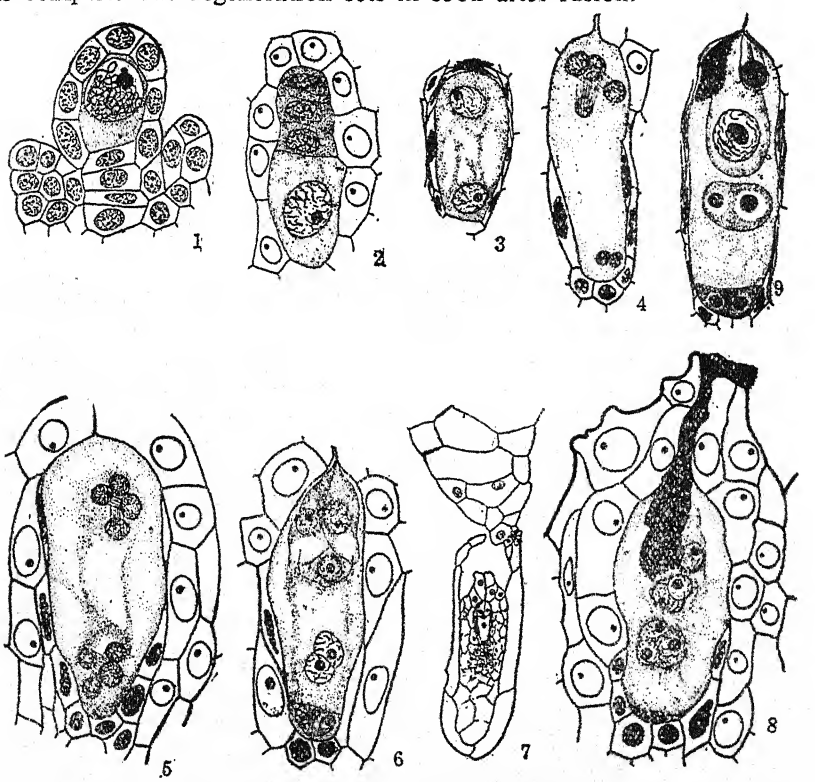
While the megaspore mother cell is in the stages of the meiotic divisions, the organisation of the inner integument consisting of two layers of cells will be complete and the outer one will have made its appearance. Simultaneously with the completion of the 8-nucleate embryo-sac, the outer integument grows beyond the inner one leaving a space between them and completes its development (Fig. 7).

The megaspore mother cell by reduction divisions gives rise to a linear row of four megaspores (Fig. 2). Chromosome counts made on the metaphase plates of the megaspore mother cell showed 41 univalents (Fig. 13). Occasionally the micropylar dyad cell divides in an oblique plane. The second reduction division follows the first immediately and the dyad cells and the four megaspores are all equal in size as soon as they are formed ; and the enlargement of the chalazal megaspore commences subsequently. In this respect this plant differs from the rest of the investigated members of its Tribe, Ophrydeæ (genera like *Orchis*, *Habenaria*, *Herminium*, etc.), wherein at the dyad stage itself there is a differentiation in size of the two cells, the micropylar cell being very much smaller than the chalazal one ; even the subsequent development of the micropylar dyad cell is most variable, sometimes not dividing at all or sometimes exhibiting a

belated development and the division product of the lower dyad cell also in the above-mentioned genera shows distinct unequality in size.

The lowermost megaspore towards the chalaza resolves itself into the mature 8-nucleate embryo-sac following the normal type of development (Figs. 3 to 6). Occasionally the mature embryo-sac contains only 6 nuclei (Fig. 4) due to the failure of division of the antipodal nuclei at the 4-nucleate condition of the sac. Under such circumstances the antipodal nuclei remain very much diminished in size. In the normal 8-nucleate sacs the antipodal nuclei organise into definite cells (Fig. 6). Synergids are organised from the division of one nucleus and the egg and the upper polar nucleus from another nucleus of the micropylar group of the four-nucleate stage.

Double fertilisation (Fig. 8) takes place quite normally and the fusion of the second male nucleus with that of the unfused polars is complete but degeneration sets in soon after fusion.



Figs. 1 to 9.—Fig. 1. Megaspore mother cell in synizesis (× 1260). Fig. 2. Linear tetrad or megaspores, the chalazal one enlarging (× 1800). Fig. 3. 2-nucleate embryo-sac (× 1260). Fig. 4. 6-nucleate embryo-sac; note the small size of the antipodal nuclei (× 1800). Fig. 5. 8-nucleate un-organised embryo-sac (× 1800). Fig. 6. Fully organised 8-nucleate embryo-sac (× 1800). Fig. 7. Longitudinal section of a seed when the embryo-sac is ready to be fertilized (× 400). Fig. 8. Double fertilization (× 1800). Fig. 9. Zygote (× 1800).

Embryo

The zygote (Fig. 9) divides by a transverse wall (Fig. 10). The subsequent development is highly variable. Sometimes the terminal cell again divides by a wall parallel to the first and thus a proembryo of a chain of three cells may be organised. The terminal cell of the proembryo divides first by a vertical wall and subsequently by walls in all planes and contributes to the organisation of the embryonal mass of cells. Usually the basal cell, sometimes also the middle cell and at other times both divide and contribute to the formation of a filamentous suspensor consisting of 4 to 5 cells (Figs. 14, 16 and 17). This organ occasionally elongates beyond the outer integument. In the majority of cases the suspensor does not extend out but becomes bent on itself and ultimately crushed between the embryonal mass and the limiting membrane of the embryo-sac (Fig. 18). In rare instances the terminal cell of the proembryo divides by a vertical wall and a suspensor as figured in Fig. 15 is organised. It is very common to find in the suspensors of the types mentioned above all stages of arrested development.

In certain ovules after the first transverse division of the zygote, the lower cell divides by vertical and later by oblique walls and forms the embryonal mass. In such cases the basal cell very slightly enlarges and remains very inconspicuous.

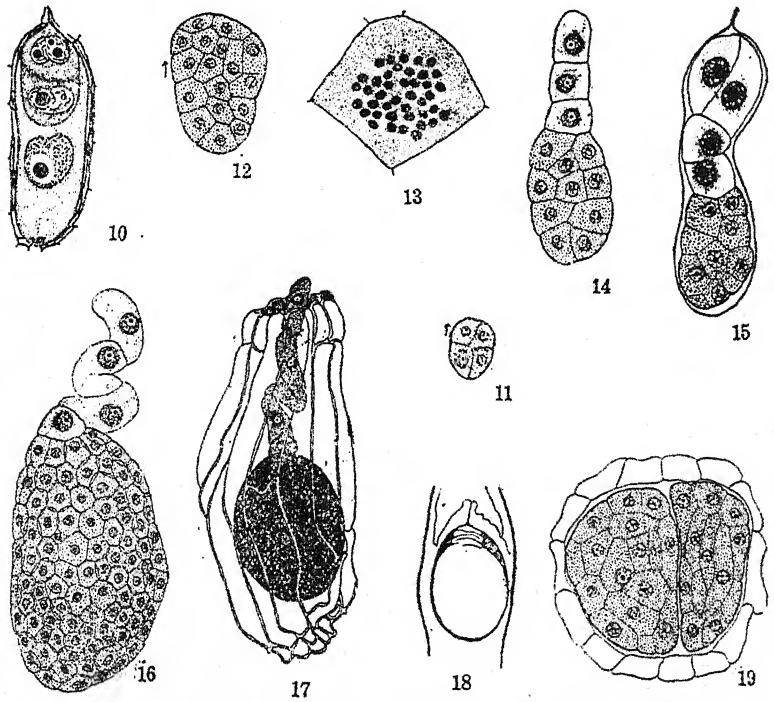
In still other ovules simultaneous divisions take place in both the cells of the two-celled proembryo in various planes to result in the mature embryo. Hence the organisation of a suspensor is completely suppressed. Some of the stages illustrating this type of development have been figured in Figs. 11 and 12.

Whatever may be the course of development of the embryo, all the mature embryos in the seeds develop to the same extent in size and differentiation ; whether it has at any time of its development no suspensor or a suspensor consisting of a single cell or of more than one cell is immaterial. It is very difficult to postulate any hypothesis for this uncertain behaviour of the suspensor organ, especially when one considers the fact that all the above-mentioned types occur in the ovules of one and the same ovary.

In this connection a comparative account of the structure of the embryo in the preceding sub-tribe Habenarieæ and the following Tribe Cypripedieæ of the present sub-tribe Diseæ (to which *Satyrium nepalense* belongs) may be considered. In all the investigated species of the genus *Habenaria* (Swamy, in press) of the preceding sub-tribe Habenarieæ, the zygote normally develops a proembryo of 3 cells as in many other orchids. The basal cell and the middle cell give rise to a filamentous suspensor of 5 to 7 cells in number. The cell of the suspensor towards the micropyle soon grows out of the outer integument, embeds itself in the placental tissue, develops lobations, and acts as an aggressive haustorium. The terminal cell of the proembryo by further divisions develop into the body of the embryo. According to Treub (1879), species of *Herminium* (the other preceding genus of the subtribe Habenarieæ) also show a very similar type of development.

In *Cypripedium* (Tribe Cypripedieæ) which is the genus next to *Satyrium*, according to Treub (1879) a rudimentary suspensor of 2 to 3 cells is developed in *Cypripedium venustum* and *C. barbatum*. In *C. parviflorum* (Carlson, 1940), the suspensor consists of a single cell " which eventually disappears ". Her figures are very vague and do not convey definitely the presence of a single-celled suspensor. In *C. reginæ* (Schnarf, 1931) a suspensor is characteristically absent.

It will be seen from the preceding account that the development of the embryo and suspensor in *Satyrium nepalense* exhibits characteristics of certain genera of its preceding subtribe Habenarieæ and the following genus *Cypripedium* of the next Tribe Cypripedieæ. Thus the plant under question may be considered as an intermediate form from the point of view of embryological evidences as revealed in the present investigation.



Figs. 10 to 19. –Fig. 10. 2-celled embryo (× 1800). Figs. 11 and 12. Stages in the development of suspensor-less embryos; the arrows point toward the micropylar pole (× 1260). Fig. 13. Metaphase plate of the megaspore mother cell during the meiotic divisions (× 3600). Fig. 14. Young embryo with a suspensor made up of three cells (× 1260). Fig. 15. Young embryo with a "T-shaped" suspensor (× 1260). Fig. 16. A normally developed mature embryo (× 1260). Fig. 17. Semi-diagrammatic representation of a mature seed with its transparent seed-coat (outer integument); the embryo in this case shows its suspensor slightly protruding out of the outer integument (× 400). Fig. 18. Diagrammatic section of an embryo showing the crushed suspensor between the embryo-sac membrane and the embryo (× 400). Fig. 19. Transverse section of a seed containing two embryos (× 400).

One instance was seen where a mature seed contained two embryos (Fig. 19) ; the exact relation of these could not be ascertained as this seed was cut transversely and this was the only one instance of polyembryony noticed.

Summary

The embryo-sac develops according to the normal type, though 6-nucleate mature sacs are of occasional occurrence. The haploid chromosome number is 41. The development of the suspensor is very variable, exhibiting all stages in the reduction of the suspensor organ and finally completely eliminating it from some embryos. When embryological evidences are taken into consideration it is manifest that *Satyrium nepalense* may be looked upon as a form holding an intermediate position between the sub-tribe Habenarieæ and the Tribe Cypripedieæ.

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A CONTRIBUTION TO THE ANATOMY OF *SALVADORA PERSICA* L. WITH SPECIAL REFERENCE TO THE ORIGIN OF THE INCLUDED PHLOEM[1]

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1. INTRODUCTION

LITTLE is known about the exact mode of origin of the included phloem in angiosperms. In their review of plant anatomy, Eames and MacDaniels (1925, p. 258) state that there are two methods by which groups of phloem cells become embedded in the secondary xylem. In genera like *Combretum* and *Entada*, the cambium, besides cutting off secondary phloem on its outer side, also gives rise to isolated strands of phloem towards the inside in place of xylem cells which are normally produced. After a brief period of such activity these cambium segments return to their normal function, and thus bury the inwardly formed phloem within the wood. In other forms, of which *Strychnos* is the best known example, the included phloem strands are originally formed by the cambium towards the outside as a part of the normal external phloem but later on new " extra-fascicular " cambial segments arise in the pericycle and unite with the general cambium cylinder so that each phloem strand becomes enclosed between a segment of the old or " residual " cambium and the new one. Of these the former becomes more or less inactive, but the latter produces xylem and phloem in the normal way so as to bury the included phloem deeper and deeper into the stem. This process is repeated several times at a number of places all around the circumference of the stem resulting in numerous " islands " of phloem within the xylem. Eames and MacDaniels (1925, p. 258) further remark that the behaviour of the cambium in such growth types has been studied in detail in but a few instances and add that probably only one of these methods (the *Strychnos*-type) occurs in all cases.

As regards *Salvadora persica*, which is the subject of the present study, Rosenvinge (1880) thought that the phloem islands (see Fig. 1) were cut off centripetally by the first method. Scott and Brebner (1889) and Chodat (1892) supported this view. A few years later Leisering (1899) ' from an examination of dozens of preparations

[1] In accordance with the terminology given by Record (1933, p. 2) the term " Included phloem " is used in this paper in place of the former " Interxylary phloem ".

of the stem' found that the phloem of the mature islands is not in direct contact with the xylem on its inner side (which ought to be the case if Rosenvinge's view is correct), but is separated from the latter by at least one and usually 2–3 layers of cambial cells. He therefore concluded that the development is similar to that in *Strychnos*, although he could not prove it with absolute certainty. Pfeiffer (1926) calls attention to the controversy regarding the origin of the phloem islands in *Salvadora* and suggests the need for a reinvestigation.

2. Material and Methods

Salvadora persica is a common tree in the North, West and South India. The material used in this study was collected from Agra, partly by Dr. P. Maheshwari and partly by Mr. B. L. Gupta and consisted of pieces of the root and stem preserved in formalin-acetic-alcohol. As the woody portion is very hard, short pieces of the stem and root were treated with dilute HF before embedding them in paraffin. Some sections were also cut on the sliding microtome without any embedding or pre-treatment. Safranin and Fast Green were used for staining. Some material of *Salvadora oleoides*, collected from Bharatpur, was also cut for comparison.

3. The Stem

General Anatomy.—In a t. s. of a moderately old stem the epidermis is seen to be replaced by a superficial layer of cork, whose outer cells seem to flake off at intervals (see Fig. 2). The phellogen arises sub-epidermally and cuts off centripetally a narrow zone of tangentially elongated cells composing the phelloderm.

The cortex is narrow consisting of only a few layers of thin-walled cells often containing druses or rhomboidal crystals. Due to the secondary changes in the stem these cells have a more or less flattened and distorted outline. An endodermis is not clearly differentiated, but the beginning of the pericycle is indicated by isolated groups of sclerenchymatous fibres connected to one another by means of thick-walled pitted cells.

At this stage the primary phloem is already crushed and almost indistinguishable. Even the secondary phloem is a narrow zone (Fig. 2), but this deficiency is more than made up by the intra-cambial phloem "islands" which are numerous and form a very conspicuous feature of the stem.

Next to the phloem is the cambium cylinder which is an actively dividing zone consisting of 4–5 layers of cells in my material. I am unable to support Leisering's statement that it forms a wide meristematic zone consisting of several layers of cells. He evidently included under the "cambium" the patches of undifferentiated parenchymatous cells cut off by it towards the inside which remain unlignified and even continue some periclinal divisions for a time (Fig. 1). It is these patches of thin-walled cells which subsequently become differentiated into the phloem islands which are dealt with in a subsequent paragraph. The wood forms the largest part of the stem. It consists of vessels,

parenchyma, and fibres. At the inner end are seen more or less radial rows of the first formed xylem vessels which protrude into the pith. Some of the protoxylem vessels are seen to be distorted and crushed owing to the enlargement of the adjacent cells of the pith. In the secondary xylem the vessel elements are numerous, rather short and narrow-lumened and occurring in groups of 2 to 8. They have simple perforations and the walls bear many alternate bordered pits. The xylem fibres are numerous and thick-walled with a few reduced bordered pits having extended slit-like apertures. The xylem parenchyma cells are particularly numerous around the vessels and phloem islands. Their walls are thin and have simple oval or rounded pits. The vascular rays are 1–7 cells wide and consist of radially elongated cells often containing squarish, rhomboidal or rectangular crystals. Such portions of the rays which pass through the phloem (normal or included) are thin-walled, but the rest consist of thick-walled pitted cells.

The pith has a more or less four-sided outline in a t.s. of a young stem. Its cells are spherical with pitted walls separated by small intercellular spaces ; some of them may contain solitary druses.

Origin and Structure of the Included Phloem.—Soon after secondary growth has commenced, the outer margin of the secondary xylem presents a more or less indented outline, which is due to the fact that at certain places the cambium cuts off towards the inside some tangentially elongated groups of thin-walled parenchyma in place of the usual lignified cells of the wood. These cells lie in perfect radial rows and differ from the cambial cells, from which they have been produced, only in having a larger radial diameter. After a short while the cambium resumes its normal activity so that the bays of thin-walled parenchyma become enclosed between the thick-walled cells of the previous and the newly formed wood. As growth proceeds some of the central cells in the islands differentiate into one or more groups of sieve tubes and companion cells (Fig. 3) but the peripheral cells still remain parenchymatous and often undergo some periclinal divisions so as to form a weak secondary cambium on the inner as well as the outer face of the phloem island (Fig. 4). In some cases the differentiation of phloem elements in the island may start even before it is actually buried into the wood, so that for a short while and at certain places phloem cells may be seen on both sides of the cambium cylinder. Only the outer of these belong to the normal phloem which is however extremely reduced in quantity, while the inner are destined to become included in the xylem. The sieve plates are usually obliquely placed. There is no special structural difference between the outer and the included phloem except that in the former some thick-walled fibres are also present. In a t.s. of a stem, about 1·3 cm. in diameter, more than 600 " islands " were counted in the wood and since *Salvadora persica* is a fairly large tree, their number must run to several thousands at the base of the stem. The inner and earlier formed phloem islands are smaller than the outer. They are further more or less circular or oval, while the outer and more recently formed groups of phloem

are very much elongated tangentially, some even becoming confluent with one another at the sides.

In the older and consequently more deep-seated islands some of the central cells get degenerated and form one or more darkly staining patches in each island (Fig. 5). As regards the cause for this obliteration Pfeiffer (1926) was unable to come to a decision and suggested two possibilities, *viz.*, that this condition may either be due to a continued activity of the primary cambium enclosed in the bundle (as in *Strychnos*) or it may be caused by a division of the elements composing the island itself. My observations lead me to the conclusion that the second view is the correct one for *Salvadora*, as also for *Leptadenia* (Singh, 1943). Cell divisions accompanied by subsequent differentiation and enlargement of the elements continue to take place in the islands for a fairly long time, but being enclosed by the thick-walled cells of the wood on all sides, the phloem cells do not find enough space for outward expansion and the whole force is therefore directed inwards resulting in a crushing of the central cells. It is also observed that the thin-walled cells of the rays which happen to pass through the older islands may similarly get crushed along with the phloem elements.

As noted before, there are often seen on the margins of the older islands a few cells which have a cambial nature. A careful study revealed that this is a secondary meristem which may appear in segments on any one side, or more than one side of the island or even all round it. If it first arises on the inner side it may give the false impression that it is an embedded segment of the original cambium ring and that the development is of the *Strychnos*-type. The activity of this cambium-like layer, and the pressure caused by it towards the centre of the island, may also be partly responsible for the crushing of cells in this region.

4. The Leaf

Petiole.—A t.s. of the petiole has a more or less circular outline with the upper side slightly flattened. The epidermis is heavily cutinised on the outside. The cortex is a broad zone of thin-walled parenchymatous cells having small intercellular spaces between them. The outer cells, forming a 2–3 layered hypodermis are polygonal, with slight thickenings at their angles and often without any intercellular spaces. There is no indication of an endodermis. The stele has an oval or flattened horse-shoe shaped form with a much greater development of the vascular tissue towards the rounded abaxial side. Small groups of fibres lie outside the isolated groups of crushed primary phloem cells. In the centre there is a very narrow flattened pith consisting of small thin-walled cells. The structure of the xylem and phloem is somewhat similar to that in the stem and it is worthy of note that the included phloem is present even in the xylem of the petiole.

Lamina.—The upper and the lower epidermis each consists of 1–2 layers of cells. Their outer walls are covered with a thin layer

of cuticle. Stomata are present on both sides. The guard cells are small and are somewhat sunken below the general level of the epidermal cells. Subsidiary cells lying parallel to the stomatal pore are clearly seen in surface preparations. The mesophyll consists of a palisade tissue on both sides enclosing a parenchymatous region along the middle line. The palisade cells are tubular and compactly packed and contain a large number of chloroplasts whose number gradually diminishes towards the interior. Interspersed amongst them are some large pouch-like cells which are considered by Sabnis (1921) to have a water-storage function but it seems that he did not notice the large spheroidal crystal in each of these cells which usually dissolves in the process of preparation of the slides. It may be added that Sabnis also failed to observe the crystals in the inner tissues of the leaf in either *S. persica* or *S. oleoides*. The cells between the two palisade regions are horizontally elongated and contain very few or no chloroplasts. Here and there in this region occur the veins and some small groups of thick-walled pitted cells which may either serve for storage of water or merely serve to give the necessary rigidity to the leaf.

The *midrib* shows a greater convexity towards the lower side. The palisade cells are continued on the upper side, but their place is occupied on the lower by large collenchymatous cells which may or may not have any intercellular spaces between them. The included phloem continues for a certain distance in the lamina, but as the amount of secondary growth decreases, it gradually disappears towards the tip.

5. The Root

General Anatomy.—The root may be diarch to tetrarch. The primary xylem strands frequently meet in the centre to form a solid xylem plate but more often a small pith is present. Secondary growth begins early and is accompanied by cork formation. The primary phloem soon becomes crushed and is unrecognisable. The secondary phloem occurs in the form of pyramidal strands composed of sieve tubes, companion cells, fibres and phloem parenchyma. In older roots it is the secondary xylem which occupies the largest proportion of the space in a t.s. Like the stem it also contains a considerable number of phloem islands scattered through it. The wood elements consist of vessels, fibres and parenchyma. There are two to four primary rays, one opposite to each primary xylem group. These are much wider than the subsequently differentiated secondary vascular rays which have the same structure as those in the stem.

Origin and Structure of Included Phloem.—The phloem islands arise in the same way as in the stem. The later formed islands are usually larger and more elongated than the first ones. Although they do not occupy any definite position in the wood, it appears that at least in later stages there is some kind of a periodicity in the activity of the cambium, which, roughly speaking, produces the thick-walled cells destined to form the wood and the thin-walled cells destined to form the included phloem, in more or less alternating layers.

The structure of the individual islands, the course of degeneration of the phloem cells and the differentiation of the secondary cambium show such close similarity with the stem that a detailed description seems unnecessary.

6. Comparison with *Strychnos nux-vomica*

While studying the origin of the phloem islands in *Salvadora*, I also cut for comparison some material of *Strychnos nux-vomica*[2] in order to gain a better insight regarding the resemblances and differences between these two forms.

My observations on *Strychnos* fully confirm those of Scott and Brebner (1889) whose account has for a long time remained the only clear exposition of the origin and nature of the phloem islands. As stated by them the islands are at first produced centrifugally as a part of the normal external phloem, but later they become bridged over by the "complementary" cambial segments, which join on to the main cambial ring (Fig. 6). These short cambial arches function normally by producing xylem towards the inside and phloem towards the outside. As a result the first formed phloem groups become buried into the wood, each having a centripetally embedded cambial segment which was once a part of the original cambium. This continues to cut off some new phloem towards the outside (but no xylem) with the result that the older phloem cells become crushed to form a sort of a cap on the outer face of each island. The islands in this species of *Strychnos* are small and circular or oval but in *Strychnos Mitscherlichii* Cockrell (1941) found that they are tangentially elongated.

It is to be noted that in *Salvadora* the condition is quite different. Unlike *Strychnos nux-vomica* the included phloem is here cut off centripetally from the normal cambial cylinder. The islands do not have any embedded cambium that can be traced back to the original cambium ring but a weak secondary cambium may occasionally arise on one or more than one side of some of the older islands. Further, the crushing of the phloem tissue takes place in the centre and not on the outside. The cap-like tissue of disorganised cells, seen in *Strychnos* is therefore not found here.

7. Conclusion and Summary

In conclusion, it may be said that the included phloem found in the wood of *Salvadora* (root as well as stem) is differentiated from the thin-walled parenchymatous cells cut off by the cambium on its inner side. Subsequently the cambium resumes its normal activity and the phloem becomes more and more deeply embedded into the wood.

If Leisering (1899) had made a developmental study of the included phloem and also compared the position of the crushed cells in the islands in *Strychnos* and *Salvadora*, he would not have come to the conclusion that they have a similar origin. As shown in this paper

[2] The material of this plant was very kindly collected for me by Mr. J. Venkateswarlu (Waltair) and Mr. M. A. K. Khalil (Dehra Dun).

it is not satisfactory to rely on the presence or absence of an embedded cambial segment on the inside of an island, for such a cambium although it seems to be characteristic of the *Strychnos*-type, may differentiate secondarily in other cases.[3] It may be pointed out that the suggestion (Eames and MacDaniels, 1925, p. 258) that of the two methods of origin of included phloem, only the *Strychnos*-type occurs in all cases and the other is probably non-existent, is incorrect.

8. Acknowledgments

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[3] Besides *Salvadora* which has been described here and *Leptadenia* (Singh, 1943), such a secondary meristem is also reported to occur around certain groups of phloem cells in the roots of some Cruciferoe (Weiss, 1883), Cucurbitaceae (Scott and Brebner, 1889), *Ipomoea batatas* (Artschwager, 1924), and *Asclepias obtusifolia* (Scott and Brebner, 1890–91).

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10. EXPLANATION OF FIGURES

PLATE III

Fig. 1. *Salvadora persica.*—A cross-section of an old stem to show the distribution of the phloem islands. × 5·5.

Fig. 2. *S. persica.*—A segment of an old stem showing the origin of the included phloem. × 66.

Fig. 3. *S. persica.*—A young phloem island showing the differentiation of sieve tubes and companion cells. × 180.

PLATE IV

Fig. 4. *S. persica.*—An included phloem island with some cambium-like cells on its outer as well as inner side. × 410.

Fig. 5. *S. persica.*—An old phloem island showing the crushing of the central cells. × 410.

Fig. 6. *Strychnos nux-vomica.*—Portion of cross-section of a stem showing the centrifugally formed phloem with an extrafascicular cambial segment on its outer side. × 290.

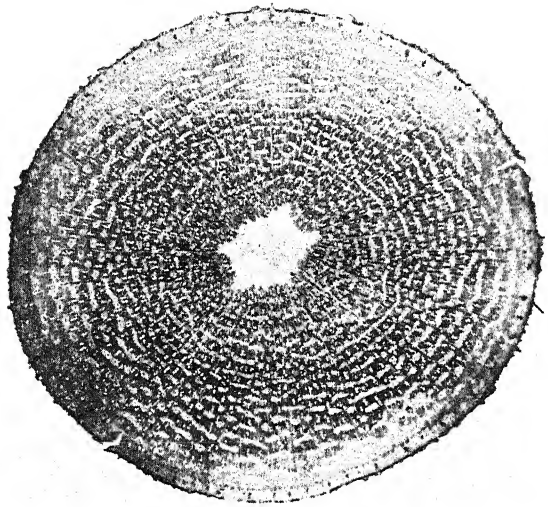


FIG. 1

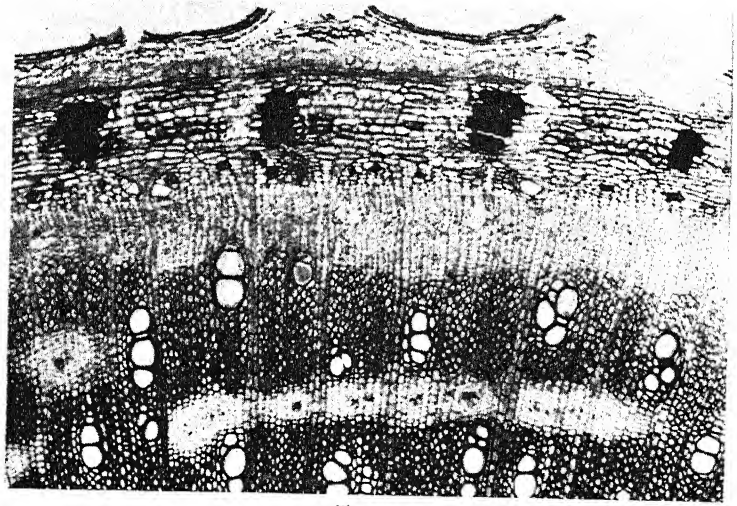
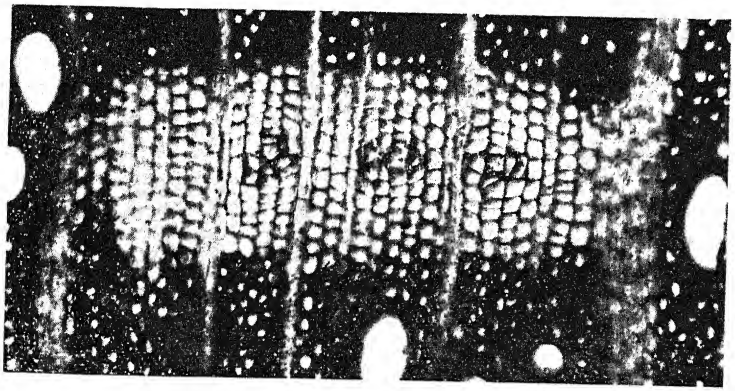


FIG. 2



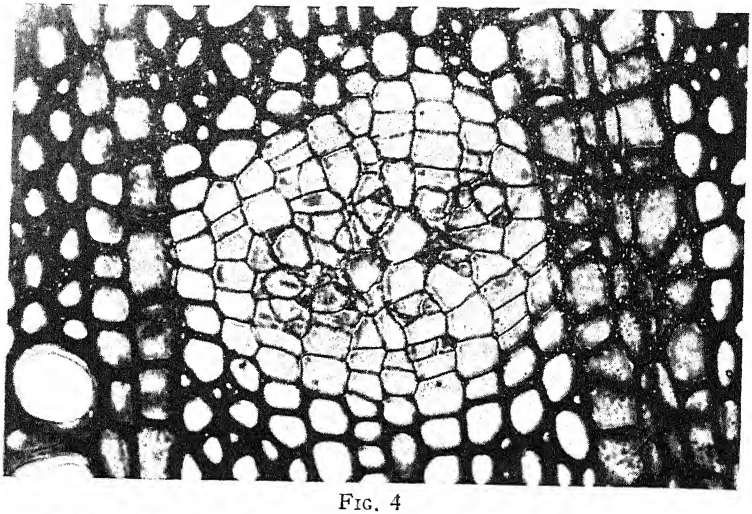


FIG. 4

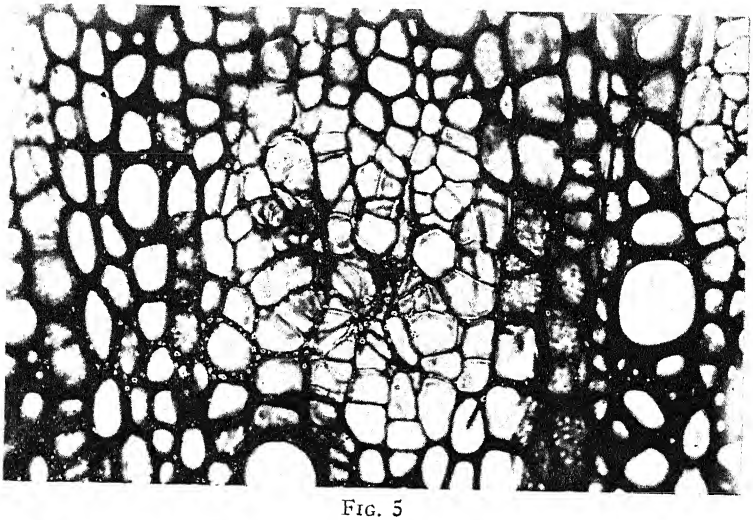


FIG. 5

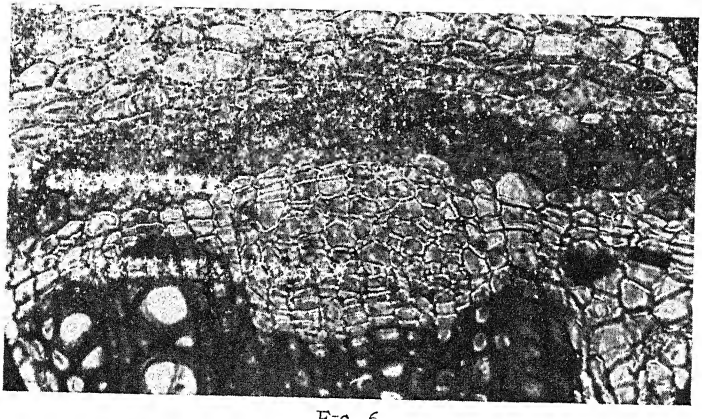


FIG. 6

THE ORIGIN OF THE HAUSTORIA IN THE OVULE OF *LOBELIA*

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Received for publication on November 28, 1943

DR. G. O. COOPER (1942) has recently published a paper on the embryology of *Lobelia cardinalis* L. in which it is stated that the synergids and antipodal cells function as micropylar and chalazal haustoria respectively.[1] The only recent work on the Lobeliaceæ and Campanulaceæ, referred to by him, is that of Kausik (1938) on *Lobelia nicotianaefolia.* There is no mention whatever of the works of Rosén (1932), Kausik (1935), Hewitt (1939) and others.

Kausik (1935, 1938) and Hewitt (1939) report that the synergids and antipodal cells in *Lobelia* degenerate at the time of fertilisation and this is in agreement with the observations made by Rosén (1932) and Safijovska (1934) on the allied family Campanulaceæ.[2] There is thus no possibility of their being responsible for the haustorial outgrowths which are clearly endospermal. In the absence of any material of *L. cardinalis* and the improbability of my being able to get it for the duration of the war, I requested Dr. S. B. Kausik of Bangalore for the loan of his preparations of *L. trigona* so that I might make an independent study of them in the light of Dr. Cooper's observations. The result of this study confirms Dr. Kausik's interpretation that the haustoria (both micropylar and chalazal) originate from the endosperm and have nothing to do with the synergids or the antipodal cells which degenerate at the time of fertilisation or shortly afterwards.

It may be added that really haustorial synergids are probably known only in a few Compositæ but even there a detailed and illustrated account of their development has never been published up to this time. In all other cases the occurrence of synergid haustoria (for a meaning of the term " haustorium " see Schnarf, 1929, pp. 352–55) is extremely doubtful. To mention a parallel instance, Heinricher (1931/32), in his monograph on the genus *Lathræa*, stated that the micropylar haustoria are derived from the synergids and the chalazal from the antipodals. This was promptly contradicted and disproved by Glišić (1932) wao made a thorough study of *Lathræa squamaria* and found that the haustoria are derived from the endosperm. The report of V. K. Srinivasan (1940) on the persistent and

[1] In the original (Cooper, p. 81) the order is given as " chalazal and micropylar " but this must be an oversight.

[2] Some authors include both the families (Lobeliaceæ and Campanulaceæ) under a common name.

presumably haustorial synergids of *Angelonia* has already been criticised by Maheshwari and Navalakha (1941) and more recently by Dr. C. V. K. Iyengar (1942). Two similar looking structures seen in *Myriophyllum alterniflorum* have been shown to be derived by a vertical division of the basal cell of the suspensor (Stolt, 1928). It is noteworthy that in this case these two cells develop even the "filiform apparatus" and hooks found in genuine synergids and the similarity is so deceptive as to mislead even the most cautious unless he has taken pains to obtain a complete series of stages in the development.

Further, it is to be noted that although one would expect *three* chalazal haustoria (granting their antipodal origin) in *Lobelia cardinalis*, actually only *two* are present and to explain this Dr. Cooper (p. 77) says that the third antipodal cell becomes "appressed" owing to the growth of the endospermal cells. Again, although double fertilisation is figured and said to take place normally there is no trace of the pollen tube in any of Cooper's figures either at the time of fertilisation or after it. On the other hand, in Kausik's (1935) figures of *L. trigona* and Hewitt's (1939) of *L. amoena* the pollen tube is quite clear and unmistakable. One would like to know how the synergids react to the pollen tube in *L. cardinalis* since in all other plants of the Lobeliaceæ and Campanulaceæ, at least one or both of them begin to disorganise on its impact.

I wish to thank Dr. Kausik for the loan of his preparations of *Lobelia trigona*. Some material of *Wahlenbergia gracilis* and *Sphenoclea zeylanica* (Campanulaceæ) as well as *Lobelia trigona*, which I recently collected from Dacca, also shows that the antipodal cells and synergids are ephemeral and the haustoria are formed from the endosperm. A more detailed account of the embryology of these plants particularly with reference to the haustoria (on whose exact origin from the endosperm there seems to be no agreement), will follow in due course, but meanwhile it is suggested that Dr. Cooper may re-examine his preparations of *L. cardinalis* in the light of the above remarks.

Summary

From a comparison of Dr. G. O. Cooper's work on *Lobelia cardinalis* with the figures and descriptions of other workers on the embryology of the Lobeliaceæ and Campanulaceæ, there seems to be no doubt that the haustorial structures which Dr. Cooper believes to have been derived from the synergids and antipodal cells are really formed from the terminal portions of the endosperm cells—a condition which is of wide occurrence in the Sympetalæ.

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DADOXYLON RESINOSUM SP. NOV. FROM THE CHHINDWARA DISTRICT OF THE CENTRAL PROVINCES

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College of Science, Nagpur

Received for publication on May 3, 1944

The block of gymnospermous wood here described belongs to the Central Museum, Nagpur. It is a solitary specimen of its kind and had been lying in the museum since the time of the late Rev. S. Hislop. The entries in the museum register indicate that Hislop had collected the fossil in the Chhindwara district but the exact locality is unknown. Nothing definite can therefore be said about the geological age of the specimen and it may be safer to discuss it separately.

Dadoxylon resinosum sp. nov.

(Figs. 1–21)

Diagnosis.—Growth rings well marked. Resiniferous tracheids mixed with medullary rays. Wood parenchyma absent. Tangential pits 1–2 *seriate, bordered, isolated, round, sometimes alternate, contiguous and hexagonal due to contact. Radial pits* 1–4 *seriate, separate and circular or contiguous and hexagonal, sometimes opposite. Pore usually circular, large. Rims of Sanio absent. Medullary rays* 1–2 *seriate,* 1–39 *cells high, average height* 22 *cells. End walls of the medullary rays mostly transverse, sometimes oblique. Pits in the field* 1–10 *(generally* 4–6*), simple, usually round.*

Locality.—Chhindwara district, C.P., India (exact locality unknown).

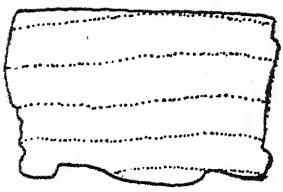
Horizon.—Unknown.

Collected by.—S. Hislop.

Reg. No.—F/149, Central Museum, Nagpur, C.P., where the type specimen is preserved.

DESCRIPTION

The specimen is a fairly well preserved block of secondary wood, 13 cm. long and 3·5 cm. × 3 cm. across the larger end (Fig. 10). The growth rings being very slightly arched (Fig. 1), the wood appears to be a piece quite far away from the pith. The autumn wood appears



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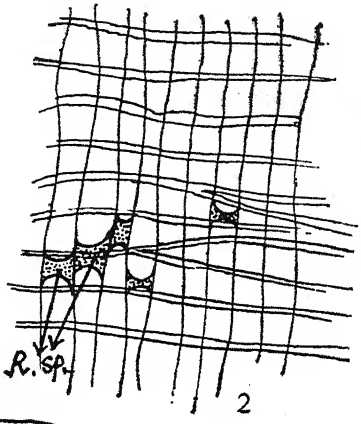

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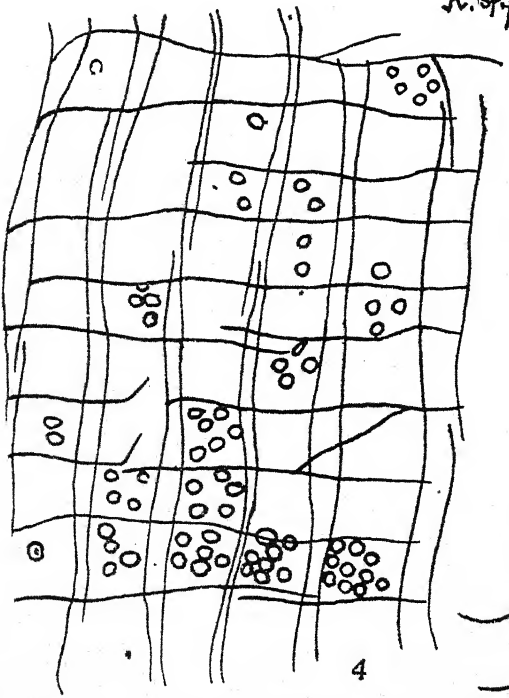

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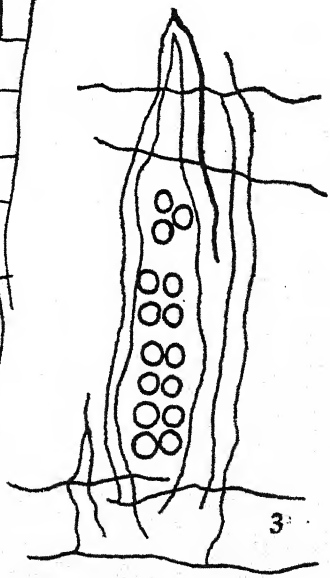

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Figs. 1—4. *Dadoxylon resinosum.*—Fig. 1. Type specimen ; transverse view of the stem showin ; growth rings. × 1. Fig. 2. Radial section. *R.Sp.*, resin spools in the medullary rays. × 100. Fig. 3. Radial section showing a single tracheid having biseriate opposite pits only. × 200. Fig. 4. Radial section showing pits in the field. × 250.

like a thin brown line about ·2 mm. thick, the spring zone being on an average 5 mm. wide.

In transverse section the growth rings are visible macroscopically (Fig. 1) as well as microscopically (Fig. 11). The autumn zone is usually represented by three or four narrow layers of tracheids (Fig. 12). The tracheids as a rule. are thick walled (average ·02 mm.) and those of the spring zone are usually isodiametric. Some of the tracheids are plugged with black stuff (? resin). The medullary rays are very crowded, being often separated by only one or two rows of tracheids. Some of the rays are also filled up with black resinous looking stuff and that gives them a prominent black appearance (Figs. 11 and 15).

The medullary rays are generally uni- or biseriate (Figs. 5 and 13). The biseriate portion is not necessarily in the middle but at or near the ends (Fig. 5, *a*, *b*) ; or portions of one ray may be alternately uni- and biseriate (Fig. 5, *d*). In some cases the medullary rays appear to be forked at the tips (Fig. 5, *c*). Their height is 1–39 cells (average 22 cells).

Tangential pits 1–2 seriate, usually round and solitary (Figs. 2 and 14), in some cases contiguous hexagonal.

The end walls of the medullary rays are mostly transverse, sometimes oblique. The resiniferous tracheids may be solitary or in groups of several (Fig. 11). The resin in most of the tracheids plugs the whole width of the cavity and sometimes it fills a fair length of the tracheid ; it always takes the form of a solid black substance (Fig. 15).

Resin plugs are also seen in some of the medullary rays (Figs. 2 and 15, *R. Sp.*).

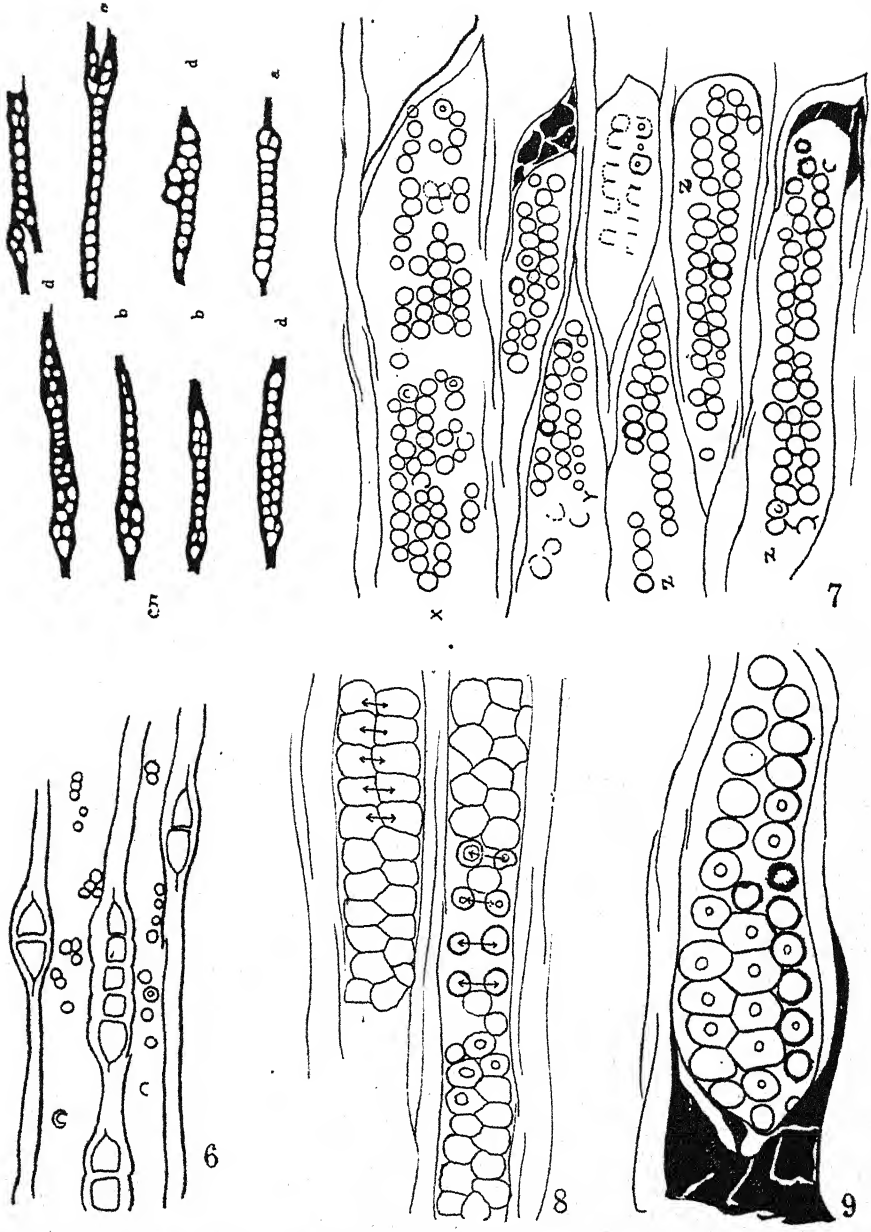
The radial pits are 1–4 seriate. The uniseriate (Fig. 17) and tetraseriate conditions (Fig. 7) are rare, the triseriate (Figs. 9 and 19) is occasional and the biseriate (Fig. 16) is the commonest. The pits may be contiguous in separate rows (Fig. 16) ; mixed uni- and biseriate conditions also occur (Fig. 18).

The pits show a tendency to lie alternately when in two or more rows (Figs. 8, 16 and 19) and in some cases within the same tracheid there may occur contiguous hexagonal and also opposite pits (Figs. 8 and 9 ; opposite pits are arrow marked). Several tracheids contain exclusively contiguous and hexagonal pits (Fig. 20).

The pits in certain tracheids are exclusively opposite (Fig. 3).

The pits in the field, 1–10 in number (Figs. 4 and 21), are mostly simple.

Average radial diameter of the widest spring tracheid (50 counts)	= ·066 mm.
Avera e radial diameter of the narrowest autumn tracheid	= ·018 mm.
Height of a vertical row of ten radial pits	= ·11 mm.
Height of the medullary rays	= 22 cells.
Dimensions of the medullary ray cells	·027 × ·033 mm.
Thickness of the tracheid wall	= ·027 mm.



Figs. 5—9. *Dadoxylon resinosum.*—Fig. 5. Various types of medullary rays as seen in tangential section. (For explanation of *a*, *b*, *c* and *d* see text.) × 38. Fig. 6. Part of a tangential section showing pits. × 60. Fig. 7. Radial section showing arrangement of bordered pits. × 150. Fig. 8. Two tracheids from a radial section having both contiguous and opposite pits. × 150. Fig. 9. A single tracheid from a radial section having triseriate contiguous hexagonal as well as biseriate opposite pits. × 225.

Systematic Position and Comparisons

As suggested by Prof. Seward (1917, pp. 249–50), the term *Dadoxylon* is used for all gymnospermous woods showing contiguous hexagonal pitting whether they are Cordaitean or Araucarian; hence the present wood is assigned to that genus. But as the age is quite unknown, it is impossible to say whether it is a Cordaitean or an Araucarian wood. It has been compared with all the southern species of *Dadoxylon* and with *Dadoxylon* (*Mesopitys*) *Tchihatcheffi*. the solitary species showing growth rings amongst the northern forms. In the following table only those characters are given in which the various species named therein differ from *D. resinosum.*

It is thus observed that the present wood differs from all the known species of *Dadoxylon.* The maximum resemblance is with *Dadoxylon Deccani* Shukla, particularly in the presence of opposite pits mixed up with contiguous hexagonal pitting; but the characters of the medullary rays, the pits on the tangential walls of the tracheids, the 2–4 seriate nature of the pits and the thick walled tracheids are enough to distinguish it. This wood, hence, appears to be a new species and it is proposed to name it as *Dadoxylon resinosum* sp. nov., the specific name referring to the abundance of resin-like substance in the tracheids.

Discussion

From the literature available on the geology of the Chhindwara district, it is gathered[1] that the only sedimentary beds found within the district are (*a*) the Lametas, (*b*) the Gondwanas, and (*c*) the intertrappeans. On the whole it seems more likely that our fossil comes from the Intertrappean series which is well known to have yielded a rich flora than that it belongs either to the Lametas or the Gondwanas of the district from which we at present know hardly any recognisable fossil woods. The Intertrappean series is now considered to be Eocene.[2]

It may, however, also be mentioned that during the Eocene age it was the Araucarian members of the artificial genus *Dadoxylon* that had persisted and not the Cordaiteans and thus the present wood may more appropriately be designated as *Dadoxylon* (*Araucarioxylon*) *resinosum* sp. nov.

Next comes the question of the systematic position of this fossil and in this connection it may be said that the occurrence of both alternate and opposite pits may be interpreted in the same way as in *Dadoxylon Deccani* Shukla. This wood may hence be considered as another connecting link between the families Araucarineæ and Abietineæ.

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[1] Medlicott, H. B. and Blandford, W. T. (1893), pp. 92–167.

[2] Sahni, B. (1934), pp. 134–136.

TABLE I.

	Medullary rays	Other characters
Dadoxylon (*Mesopitys*) *Tchihatcheffi*[1]	Markedly low	
D. Zalesskyi Sahni[2]	Uniseriate, low	1–5 rows of pits. Field pits without a border.
D. Krauseli Sahni & Singh[3]	1–20 cells high	Rims of Sanio present.
D. lafoniense Halle[4]	Low 1-ser., pits 2-ser.	Secretory pits present.
D. indicum Holden[5]	Low, uniseriate	1–2 rows of pits. Pits having a tendency to fuse and unite to form a single big pit.
D. bengalense Holden[6]	Uniseriate, 1–20 cells high	2–7 field pits forming a group.
D. (*Araucarioxylon*) *rajmahalense* Sahni[7]	Uniseriate, 1–20 cells high	2–3 seriate pits.
D. α Sahni[8]	Uniseriate, low	
D. β Sahni[9]	1–24 cells high	Pits 2–3 seriate. Growth rings not well marked.
D. parbeliense Rao[10]	1–6 cells high	Pits 1–5 seriate. Field pits 8–9.
D. teilhardi Sze[11]	3–20 cells high	Biseriate pits. Field pits 8–9.
D. rhodeanum Göppert[12]	Mostly uniseriate, 2–49 cells high	2–4 rows of pits. Annual rings absent.
D. Deccani Shukla[13]	1–16 cells high	1–2 seriate pits. Field pits 1–6. No tangential pits.
D. Bakeri Seward & Walton[14]	1–20 cells high	
D. sp. Warren[15]	1–20 cells high	
D. sp. Walton[16]	1–25 cells high	
D. angustum Felix[17]	1–25 cells high	
D. Arberi Walton[18]		

[1] Frentzen, K. (1931), pp. 617–24.
[2] Sahni, B. (1933), p. 421.
[3] Sahni, B. and Singh, T. C. N. (1926), pp. 103–12.
[4] Halle, T. G. (1911), p. 64.
[5] Holden, R. (1916), p. 318.
[6] Holden, R. (1916), p. 322.
[7, 8, 9] Sahni, B. (1931), pp. 69, 71, 72 respectively.
[10] Rao, H. S. (1936), p. 174.
[11] Sze, H. C. (1934).
[12] Gothan, W., und Sze, H. C. (1933), pp. 87–103.
[13] Shukla, V. B. (1938), pp. 359–62.
[14] Seward, A. C., and Walton, J. (1923), pp. 313–33.
[15] Warren, E. (1912).
[16] Walton, J. (1925), p. 2.
[17] Halle, T. G. (1911), p. 68.
[18] Walton, J. (1925), p. 2.

SUMMARY

The present wood is the second petrified gymnospermous wood to be described from the Chhindwara district. The exact locality and therefore the geological age is unknown. It is, however, quite possible that this wood might have come from the Intertrappean beds of that district, which are now considered to be of Eocene age. Comparisons have been made with all the known *Dadoxyla* of the Gondwana type, including *Dadoxylon* (*Mesopitys*) *Tchihatcheffi*, but as it differs from all of them, it has been referred to a new species *D. resinosum*. The specific name refers to the abundance of a resin-like substance in the tracheids which seems to be a characteristic feature of the wood. As this species, like *D. Deccani* Shukla, shows a combination of alternate and opposite pits, it may also be considered as a connecting link between the families Araucarineæ and Abietineæ.

ACKNOWLEDGMENT

The author wishes to express his deep sense of gratitude to Prof. B. Sahni, F.R.S., for his very kind help, keen interest and constant guidance in the progress of this work.

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EXPLANATION OF PLATES

Plate V

Fig. 10. *Dadoxylon resinosum* sp. nov. Type specimen (× 9/13).

Fig. 11. Type specimen. Transverse section showing a growth ring (× 30)

Fig. 12. Type specimen. Transverse section showing an autumn zone and parts of two spring zones on the either side (× 200).

Fig. 13. Type specimen. Tangential section showing uni- and biseriate medullary rays (× 100).

Fig. 14. Type specimen. Tangential section showing bordered pits on the tangential walls of the tracheids (× 290).

Plate VI

Fig 15. Type specimen. Radial section showing tracheids and medullary rays plugged with condensed black resiniferous substance (× 15 0)

Fig. 16. Type specimen. Radial section showing biseriate condition of pits in the tracheids (× 230).

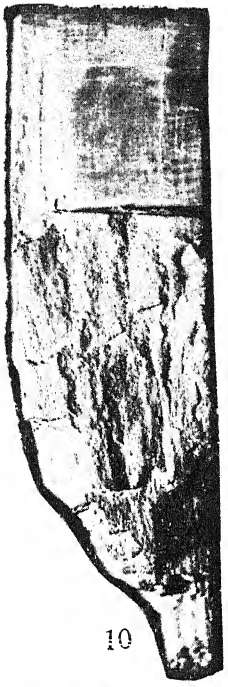
Fig. 17. Type specimen. Radial section showing uniseriate condition of pits in the tracheids (× 130).

Fig. 18. Type specimen. Radial section showing tracheids with uni- and biseriate pits in the same trracheid (× 150).

Fig. 19. Type specimen. Radial section showing a tracheid with triseriate pits (× 230).

Fig. 20. Type specimen. Radial section showing tracheids with contiguous hexagonal pitting (× 230).

Fig. 21. Type specimen. Radial section showing pits in the field (× 410).



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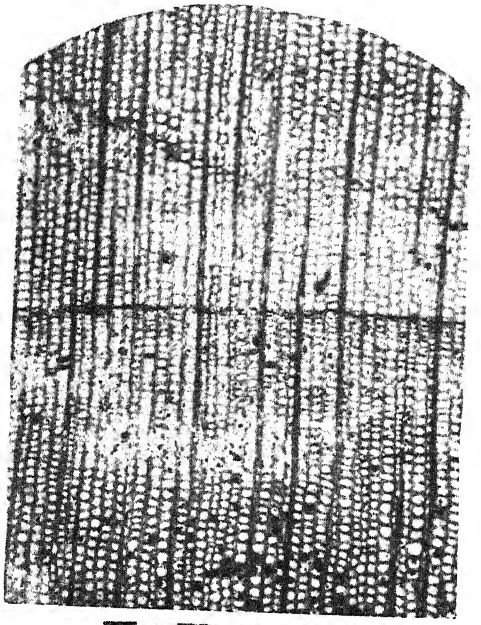

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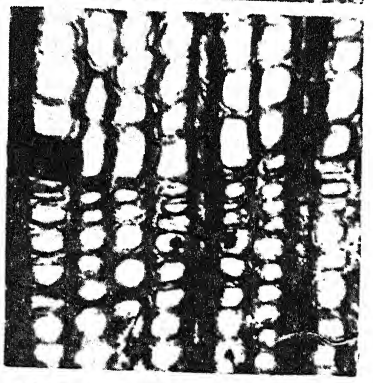

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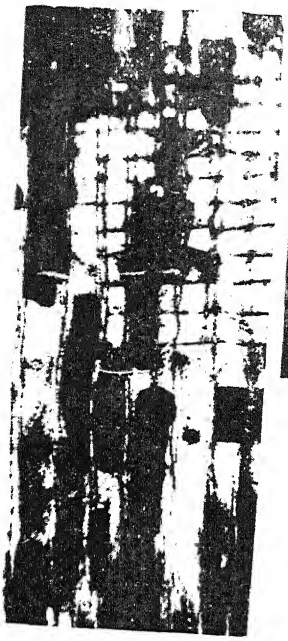

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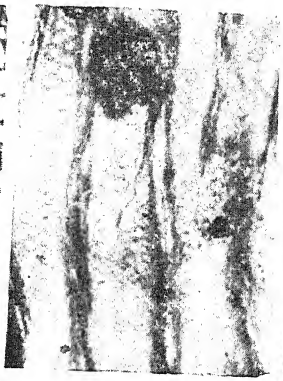

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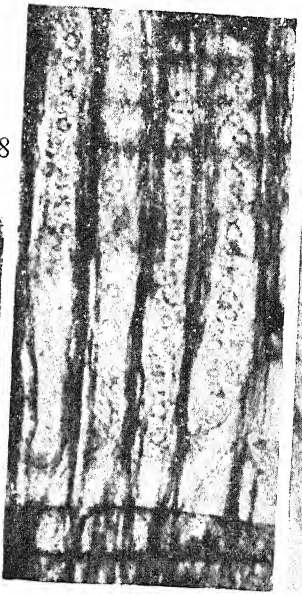

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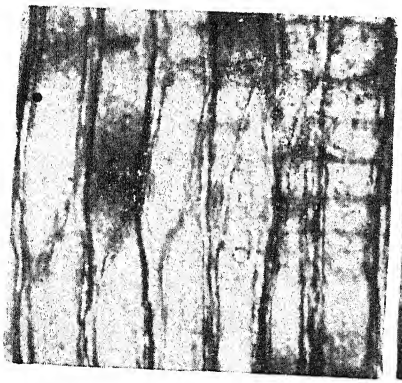

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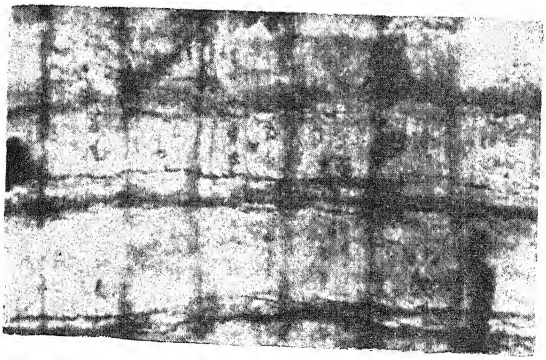

21

PHYSIOLOGY OF *CERCOSPORA SESAMI* ZIMM.

By S. Chowdhury

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I. Introduction

A leaf spot disease of *til* (*Sesamum indicum*) caused by *Cercospora sesami* Zimm. has been prevalent in Sylhet and its neighbourhood for some years. It has been found to cause considerable damage. This disease has been known also to occur in other parts of Assam wherever *til* is grown and has also been reported from Bombay by Uppal, Patel and Kamat (1935).

In this paper are reported the characteristics of the disease and the results of the studies on the morphology and physiology of the fungus. These studies throw light on the role of external factors on the nature and spread of the disease.

II. Symptoms of the Disease

The disease usually makes its appearance just at or before the time of flowering. But sometimes plants a month old have also been found attacked. The attack is more severe in the later stages.

The disease manifests itself generally as small light brown spots 2–5 mm. in diameter on the leaves. These spots are at first more or less roundish, but later on become irregular in outline and occasionally several coalesce forming irregular spots often as big as 5–15 mm. in diameter. The spots are found on both surfaces of the leaf. The colour of the spots which is at first light brown changes to a darker colour with the formation of the conidiophores and the conidia. The leaf tissue around the spots very often loses the normal green colour and takes a yellowish hue.

On the petiole the spots appear along its length; they are elongated and of varying lengths. They are at first light brown as on the leaves but gradually become dark.

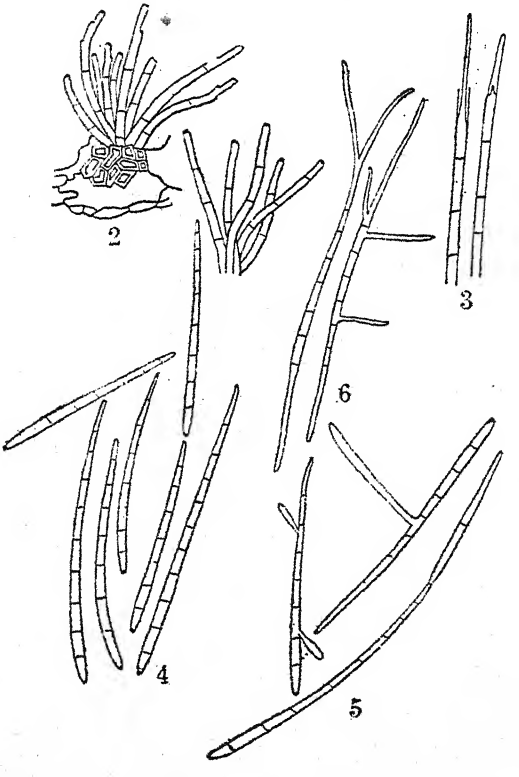
The stem as a rule is much less infected than the leaves. In shape and colour the spots on the stem resemble to a very great extent those on the petiole. Sometimes the whole stem dries and the plant droops down.

Lesions also appear on the pods and they are often numerous. They are more or less circular and measure from 1–7 mm. in diameter. In early stages the spots are brown in colour but in advanced stages they become black and often the pod is destroyed by the parasite. Fig. 1 shows the symptoms of the disease.

III. Morphology of the Parasite on the Host

Mycelium.—The mycelium of the fungus within the host is composed of irregularly septate thick-walled light brown hyphæ. Younger hyphæ are thin-walled and sparsely septate while the older ones are brown, thick-walled, tortuous with septa at short intervals. Mycelium is usually intercellular but in old, disintegrated tissues it also becomes intra-cellular. Hyphæ collect in the air spaces under the stomata and form stromatic masses giving rise to conidiophores which emerge through the stomata.

Conidiophores.—Conidiophores are light brown when young and dark brown when mature. They are produced in clusters, the number in each cluster varying from 5–10. They emerge through the stomata and clusters may be found to come out through most of the stomata. Each cluster has at its base a stromatoid structure (Fig. 2) made up of brown coloured cells. From these the conidiophores originate.



Figs. 2–6. *Cercospora sesami.* –Fig. 2. Conidiophores from nature. Fig. 3. Germination of conidiophores. Fig. 4. Conidia from nature. Fig. 5. Conidia giving rise to conidiophore and secondary conidia. 6. Germination of conidia. × about 600.

Each conidiophore is uasully unbranched but branched conidiophores are by no means rare. They measure from 38·5 to 67·5 $\mu \times 4\mu$ and are usually 0–3 septate. At the apex each conidiophore presents a number of characterisitc bends. At each bend there is a scar left by a spore. The scar appears as a slightly thickened area and looks darker under the microscope. The number of scars represents the number of spores produced and 1 to 6 scars may be noticed on a single conidiophore. The conidia are developed at the tips of conidiophores. After one conidium has been formed the stalk elongates past the conidium producing another again and thus the bends are formed. Conidiophores or their pieces readily germinate in tap water sending out germ-tubes from both ends as well as from sides near the septa (Fig. 3).

Conidia.—The conidia are sub-hyaline to very light yellowish, 7–10 septate and elongated, broader at the base and tapering towards the apex (Fig. 4). At the base of the conidium a scar is present showing the place of attachment to the stalk and this appears as a darker area. They measure 88–136 μ in length and 3–4 μ in width. The conidial wall is usually smooth but rarely constrictions are formed at the septa. Old conidia are of a brownish tint and sometimes one or two of their cells are found shrunken.

IV. PARASITISM

Single spore culture of the fungus was obtained by the usual plating method, and a series of inoculation experiments were carried out both in the field and the laboratory on plants of *Sesamum indicum* by placing spores and mycelium on unwounded host parts or by spraying with spore suspensions in sterile water. The results show that the fungus can attack every part of the host plant. The details of the inoculation experiments are summarised in Table I.

TABLE I

Summary of the results of inoculation experiments on Sesamum indicum *by* Cercospora sesami

Parts of the host inoculated	No. of inoculations	No. of plants infected	No. of controls kept	No. of controls infected
Leaves —				
Upper surface ..	86	71	39	Nil
Lower surface ..	78	62	42	,,
Petioles	27	25	22	,,
Stems	45	39	29	,,
Pods	48	41	32	,,

It will appear from Table I that the fungus is a parasite capable of attacking all parts of the plant. It was also observed that infection takes place readily both with mycelium and spores. In the case of mycelium as inocula infection takes place within 48 hours and with spore infection spots begin to appear after 4 to 6 days.

The fungus was isolated in all cases from the infected plants and it agreed with the parasite with which the infection was done.

V. Studies under Conditions of Artificial Growth

A. Macroscopic characters

(i) *Growth on different media*

The fungus was cultivated on a large number of media and on all the media the fungus made a good growth except on Brown's synthetic agar in which staling took place. The cultural characteristics of the fungal growth on the different media at 25° C. are recorded below :

Oat meal agar.—Abundant woolly aerial mycelium, colour white at the centre, light forgetmenot blue elsewhere and on the margin. Substratum light greyish indigo in the centre, edge light sky blue.

Dox's agar.—Abundant aerial mycelium slightly felty, dirty, very light green, edge light lilacy white. Substratum dark grey green in the centre, edge dark sheet grey.

Hopkins' agar.—Abundant woolly aerial mycelium, white to very pale yellowish, sparse on the edge. Substratum light cinnamon in the centre, then surrounded by a zone of dark blackish green.

Richards' solution agar.—Aerial mycelium profuse, woolly, light sky coloured white at places. Substratum dark cypress green.

Coons' agar.—Aerial mycelium abundant, cottony, very light purplish white, edge light greyish indigo. Substratum olive grreen.

(ii) *Depth of medium*

The effect of depth of medium upon linear rate of growth of the fungus was studied on Dox's agar and oat agar at 25° C. Petri dishes of equal size were supplied with 10, 20 and 40 c.c. of the media. Triplicate plates were used for each series. The inoculated plates were incubated at 25° C. and the diameter of the colonies was measured after seven and fourteen days. The details are given in Table II.

Table II

Influence of depth of medium on linear rate of growth of C. sesami

Media	Amount of media	Days	
		7	14
	c.c.	mm.	mm.
Dox agar	10	31·5	50·5
	20	35·5	57·0
	40	38·0	65·0
Oat agar	10	30·0	55·0
	20	31·5	60·9
	40	34·0	70·2

It will be obsreved from Table II that the linear rate increases with the increase in the depth and amount of nutrient. Similar results were obtained by Mitra (1931) on species of *Helminthosporium* and by Singh (1934) on *Cercospora indica* although Coons and Larmer (1930) found in the case of *Cercospora beticola* that the depth of media had very little influence on growth. It appears therefore that in carrying out determination of growth rate under different environmental conditions the depth of medium should be uniform.

(iii) *Light*

Effect of alternate light and darkness, continuous light from 100-watt electric lamp and continuous darkness on linear rate of growth was carried out on Hopkins' agar. To study the effect of alternate light and darkness inoculated plates were placed in front of a window at room temperature. Alongside of them cultures to be kept in continuous darkness were put inside big blackened cover dishes after wrapping them in black paper. Cultures to be kept in continuous light were placed in front of a 100-watt electric bulb in a darker corner of the same room. The diameters of the different colonies were measured after 10 and 20 days and the results are recorded in Table III.

TABLE III

Effect of alternate light and darkness, continuous light and continuous darkness on Hopkins' agar on the growth of C. sesami

	10 days	20 days
	mm.	mm.
Alternate light and darkness	22·0	34·4
Continuous darkness	18·2	24·0
Continuous light	12·6	18·3

It will be evident from Table III that the rate of linear growth is greater in alternate light and darkness, less in continuous darkness and least in continuous light. The retarding effect of continuous darkness and continuous light becomes more evident with time. Similar results were obtained with species of *Helminthosporium* by Mitra (1931).

(iv) *Humidity*

A study of the growth rate of the fungus under different relative humidities was made in accordance with the method of Stevens (1916). Cultures were exposed to atmosphere with different degrees of humidity by using sulphuric acid of varying specific gravities. Sterilized dishes of uniform size were used for the purpose and a known volume of the acid was put in each to fill about one-foruth of the volume. Petri dishes of uniform size in which equal amounts of the same medium had been poured and inoculated with the fungus were fixed with gelatine solution to glass panes big enough to fit on the top of the containers. The lids of the petri dishes were removed and the glass panes with dishes were sealed with vaseline to the containers

According to this arrangement the surface of the medium on which the fungus was growing was facing downwards exposed to the acid solutions, exerting known vapour pressure in the manner figured and described by Paul (1929). The rate of growth was measured and the data of the measurements, which are the average of two experiments, each running in triplicate, are given in Table IV.

TABLE IV

Linear rate of growth of C. sesami *in varying atmospheric humidity*

Relative humidity	Growth in millimeters		
	6th day	12th day	15th day
50 per cent.	24·0	26·1	27·4
70 ,, ...	22·6	28·5	31·9
78 ,, ...	19·8	32·7	36·0
92 ,, ...	17·2	36·9	47·3
100 ,, ...	12·9	25·2	36·0

The results presented in Table IV show that the fungus tolerates a wide range of humidity from 50–100 and the best growth is in an atmosphere of 92 per cent. humidity though the growth is faster at lower humidities during the first few days. In an atmosphere fully saturated with water vapour the growth is slow.

(v) *Temperature*

The linear rate of growth of *C. sesami* was studied on Hopkins' and Richards' solution agars at various temperatures. The experiment was carried out in selected Petri dishes of uniform size into which equal amounts of the medium were poured. All the dishes were inoculated at the same time and kept at various temperatures in darkness. The experiment was run in triplicate and repeated twice. The diameters of the growing colonies were measured from time to time and the data obtained are presented in Figs. 7, 8 and 9.

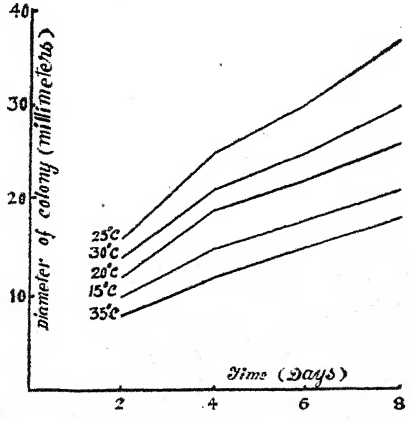


Fig. 7. Temperature relationship of *C. sesami* on Richard's solution agar

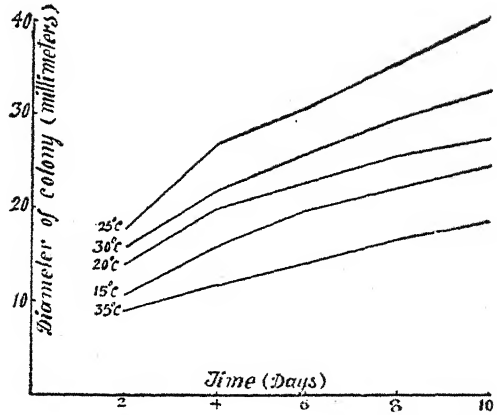


Fig. 8. Temperature relationship of *C. sesami* on Hopkins' agar

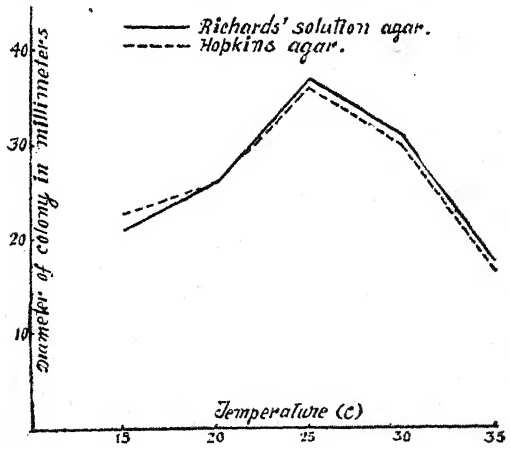


Fig. 9. Eight days' growth of *C. sesami* on Richards' solution agar and Hopkins' agar at various temperatures

It will be seen from these figures that the fungus grows well between 20° C. and 30° C. and that temperatures above and below are detrimental to the growth of the fungus. It will also be noticed that the optimum temperature for growth lies very probably between 25° C. and 30° C. At higher temperatures an abnormal type of growth takes place and the colony becomes pale and forms a lot of felty aerial mycelium.

(vi) *Concentrations*

The fungus was grown on 10 N, 5 N, 2 N, N, N/2, N/5, N/10, N/20, N/50 and N/100 concentrations of Coons' solution in flasks of uniform capacity containing 100 c.c. of the medium. Inoculated flasks were kept at 25° C. and the mycelium was filtered after 31 days and

the dry weight of the fungus determined. The experiment was run in triplicate and the data obtained, which is the average of the three, are shown in Table V.

TABLE V

Average dry weight of the mycelium in gms. of C. sesami *at various concentrations of Coons' synthetic solution*

Concentrations	Average dry weight of the mycelium in gms.
10 N	0·5672
5 N	0·2706
2 N	0·1461
N	0·0678
N/2	0·0281
N/5	0·0102
N/10	0·0017
N/20	0·0013
N/50	0·0009
N/100	0·0003

From the data presented in Table V it will appear that the dry weight of the mycelium increases in direct proportion to the increase in concentration of the medium and decreases with the decrease in the concentration of the normal solution. The results obtained agree with those of Moore (1924) and Brown (1925).

(vii) *Importance of the different constituents of Richards' solution*

The importance of the different constitutents of Richards' solution on the growth of the fungus was determined by eliminating from the culture solution each salt one by one. The data so obtained are presented in Table VI.

TABLE VI

Average dry weight of mycelium of C. sesami *grown on Richards' solution and those lacking in one of its constituents at* 25° *C. after* 30 *days*

Media	Dry weight of the mycelium in gms.
Normal	0·314
No $FeCl_3$	0·261
No KH_2PO_4	0·163
No $MgSO_4$	0·132
No KNO_3	0·034
No sugar	..

It will appear from Table VI that sugar is the most important constituent of the medium. Without sugar there was practically no growth. Next to sugar KNO_3 seems to be most essential. The absence

of ferric chloride was much less felt than either $MgSO_4$ or KH_2PO_4. In normal medium the fungus showed the best growth.

(viii) *Zonation*

Concentric zones were noticed in all the plates, faintly in some and distinctly in others. Zones have been noticed in the growths of other fungi and the formation of these has been attributed to various causal agencies, ' to light relation, to nutrients, to agencies other than light probably food, to resting periods and to mycelial crowding ' (Stevens and Hall, 1909). Brown (1925) has observed in the growth of certain strains of *Fusarium* definite series of rings corresponding to the alteration of day and night. Bisby (1925) in his review of the literature on the subject has stated that different fungi gave variable results.

In this fungus the number of zones differed in different media. They were most sharply defined on oat meal agar, Richards' solution agar and Coons' agar. On Hopkins' and Dox's agar no zonation could be noticed.

With regard to this fungus it was also found that the formation of the zones is due to alternation of light and darkness. The zones were absent in cultures kept in continuous darkness and in continuous light but quite distinct in those kept outside exposed to light during the day and to darkness during the night.

Temperature has also been noticed to play an equally important part in zone formation. This fact has also been observed by Christensen (1926) in the case of *Helminthosporium sativum* and by Mitra and Mehta (1934) in the case of *H. nodulosum*. Bisby (1925) obtained similar results from *Fusarium discolour sulphureum* and Coons and Larmer (1930) from *Cercospora beticola.*

Zonation can be induced by fluctuating of temperature within a certain range. The data of zonation in relation to temperature are given in Table VII.

TABLE VII

Relation of temperature to zonation in C. sesami *in darkness in Dox's agar*

Temperature °C.	Zonation	Remarks
10	Nil	Constant
20	,,	,,
30	,,	,,
35	,,	,,
20-30	Traces	Alternating
20–25–30	Good	,,
35–38	Nil	,,

In *C. sesami* at a constant temperature zones either failed to appear or were only very faintly visible. The alternation of temperature within 20°–30° C. resulted in the appearance of zones, but above or below this range zonation could not be induced.

B. Microscopic characters

(i) *Character of the mycelium*

The mycelium when young is septate at long intervals and is sub-hyaline ; with age it becomes light brown to brown and septa appear at short intervals. In older cultures it becomes dark brown, highly tortuous and toruloid.

(ii) *Factors affecting sporulation*

(*a*) *Light.*—Continuous light or darkness has been found to inhibit sporulation. Cultures kept in alternate light and darkness (day and night) have been found to sporulate earlier and more copiously than those kept in continuous light or darkness. It was further observed that when the fungus was grown in petri dishes in darkness and after a few days the growth was exposed to light the formation of spores was stimulated.

(*b*) *Humidity.*—Conditions of high humidity also seemed to favour sporulation. Plates with growing cultures of the fungus held upside down over water surface and various percentages of sulphuric acid showed differences in sporulation. Conditions of high humidity from 80 to 100 per cent. favoured sporulation while at a lower humidity its formation was entirely suppressed.

Sometimes sporulation has been found to be copious in cultures saturated with water. When bits of agar containing mycelium of the fungus were placed in watch glasses and saturated with distilled water copious sporulation took place.

(*c*) *Temperature.*—Change of temperature was found to have a stimulating influence on sporulation. Sporulation was accelerated by growing the fungus at low temperatures (10°–15° C.) and after about 10 days' growth exposing the culture to higher temperatures (30° C.).

(*d*) *Media.*—Other factors being the same sporulation varied on different media. In general sporulation was more liberal when the fungus was grown on meal agars than on synthetic media. The most copious spore production, however, took place when the fungus was grown on sterilized *Sesamum* stems in tubes.

TABLE VIII

Sporulation of C. sesami *on different media*

Sterilized *Sesamum* stems	.. Very good
Oat meal agar	.. Good
Maize meal agar	.. Good
Hopkins' agar	.. Moderate
Dox's agar	.. Moderate
Browns' synthetic agar.	.. Poor

(*e*) *Wounding.*—In certain instances sporulation is accelerated in a culture by local wounding. Petri dish cultures which were wounded here and there were found to sporulate fairly strongly along the lines of wounding.

(iii) *Size and septation of conidia*

The size and septation af conidia have been found to vary under different environmental factors, the most important being temperature, relative humidity and media.

(*a*) *Temperature.*—An important factor in affecting the size and septation of conidia is temperature. The shape of the conidia is, however, not much affected. The spore size and septation at different temperatures are given in Table IX.

TABLE IX

Variation in size and septation of conidia of C. sesami *on Hopkins' agar at various temperatures*

Temperature	Length		Width		Septation			
	Range μ	Mean μ	S.D. μ	Range μ	Mean μ	Range	Mean	Mode
C								
20	64–015	87·64 ±0·29	7·5 ±0·207	2·5–4	3·5	6–10	8	8
25	86–130	109·50 ±0·38	9·9 ±0·273	2·5–4	3·4	7–10	9	8
30	77–123	99·60 ±0·39	10·1 ±0·279	2·0–3·5	3·4	7–10	8	7
35	64– 96	79·50 ±0·38	8·5 ±0·275	1·5–3·0	2·5	5– 8	6	5

From Table IX it will be seen that the mean length of the conidia is the greatest at 25° C. and the least at 35° C.; conidia formed at 20° C. and 30° C. have their mean length less than those formed at 25° C. but more than those formed at 35° C. The mean width decreases with the rise in temperature and the mean septation is the greatest at 25° C., same at 20° C. and 30° C. and the least at 35° C.

(*b*) *Relative humidity.*—Welles (1925), Klotz (1923) and Sundararaman and Ramakrishnan (1928) and Ramakrishnan (1931) found on different species of *Cercospora* that remarkable increase in size and septation of conidia takes place at high humidities. An experiment was carried out by incubating infected leaves at relative humidities of 47, 70·4 and 100 per cent. and after forty hours a count of 300 conidia were made at random. The results are recorded in Table X.

TABLE X

Effect of different relative humidity on size and septation of conidia of C. sesami

Relative Humidity	Length			Width		Septation		
	Range μ	Mean μ	S.D. μ	Range μ	Mean μ	Range	Mean	Mode
47·0	86–125	102·93 ±0·33	8·7 ±0·24	3–4·0	3·5	7–10	8·5	8
70·4	96–169	128·21 ±0·59	15·3 ±0·42	3–3·5	3·2	7–12	10·5	10
100·0	86–245	157·86 ±0·97	23·5 ±0·69	3–3 5	3·2	7–17	13·0	11

It will be seen from Table X that the average length of conidia increased and the average width decreased with the rise in relative humidity. The number of septa also increased with the increase in the relative humidity.

(*c*) *Media.*—The size and septation of conidia depend on the nature of the medium on which the fungus is cultivated. Conidia measurements were made from fifteen days' old cultures grown on Dox agar, sterilized *Sesamum* stems and oat meal agar and are shown in Table XI.

TABLE XI

Variation in size and septation of conidia of C. sesami *on different media*

Media	Length			Width		Septation		
	Range μ	Mean μ	S.D. μ	Range μ	Mean μ	Range	Mean	Mode % Septation.
Sesamum stem	87–135	110·88 ±0·40	10·3 ±0·28	3 4	3·4	7–10	9	9
Oat agar ..	80–127	105·0 ±0·38	10·0 ±0·27	2·5–3·5	3·0	6– 8	7	7
Dox agar ..	80–120	101·76 ±0·42	10·9 ±0·42	2·5–3·	3·0	6– 8	7	6

It will be seen from Table XI that under identical conditions the average length of the conidia is the greatest on *Sesamum* stems and the least on Dox agar ; the average width was same in Dox and oat meal agars and slightly more on *Sesamum* stems. Number of septa was the greatest on *Sesamum* stems and same on Dox and oat meal agars.

(iv) *Secondary spores*

The formation of secondary conidia from primary conidia often takes place both in nature as well as in culture. In some cases the first formed conidium produces a second one at its tip, while in others lateral conidia are formed from the sides of the basal cells of the larger conidia (Fig. 5). The secondary conidia so formed are small and usually non-septate, rarely one septate.

(v) *Spore germination*

Under the most favourable conditions conidia may germinate within 4 to 6 hours but they usually require longer and some do not germinate for days, although to all appearances conditions are favourable.

The conidia readily germinate in tap watet within 4–6 hours. Germ tubes are given off (Fig. 6) from both ends of spore as well as from sides near the septa. At times two germ tubes originate from

a single cell of a conidium. Conidiophores and even bits of them also readily germinate in tap water (Fig. 3).

The time required for the germination of spores varies with the culture media used, the age of the spores, temperature, hydrogen-ion concentration and humidity.

(*a*) *Sugar.*—Sugar solutions have been found to exert a favourable influence on the germination of spores. Spores which germinated slowly in distilled water and tap water did so more quickly when placed in sugar solutions ; a higher percentage of spores germinated and the germ tubes also attained greater length. The results obtained are given in Table XII.

TABLE XII

Effect of sugar on the germination of spores of C. sesami

Media	After ten hours	
	% of germination	Av. length of germ tube s(μ)
Distilled water	25·0	12·6
Tap water	32·5	20·9..
5% glucose solution ..	86·9	45·2
5% succrose solution ..	88·4	44·0

(*b*) *Temperature.*—Spores were taken from a 15 days' old culture on *Sesamum* stems and spore suspensions made in sterilized distilled water. A drop of the spore suspension was placed in the centre of a cover slip and inverted over Van Tiegham rings containing a few drops of distilled water. These were then exposed for 12 hours at 20° C., 25° C., 30° C. and 35° C. temperatures. Percentage germination of spores and the average length of the germ tubes for each temperature were determined and the results are recorded in Table XIII.

TABLE XIII

Germination of conidia of C. sesami *at various temperatures*

Temperature ° C.	% of germination	Av. length of germ tubes (μ)
20	72	37·5
25	80	62
30	61	51·0
35	32	19·1

It will be found from Table XIII that the percentage of germination as well as the average length of germ tubes is the greatest at 25° C.

(*c*) *Humidity.*—The effect of various percentages of relative humidities on the germination of the spores of *C. sesami* was determined according to the method of Lesage (1895). The principle involved is that the saturation of the air above a given solution of sodium chloride varies inversely with the concentration of the salt dissolved therein and the humidity is said to remain constant though the

temperature may vary. Moisture free sodium chloride was taken and solutions containing 4·5 to 25·0 gms. per 100 c.c. of water were prepared and 25 c.c. of the solution was put in dishes of 6 cm. ×3·5 cm. A drop of distilled water containing a spore suspension was spread on the underside of each of the lids and dried *in vacuo* over calcium chloride. The lids were then placed over the dishes and sealed with a mixture of paraffin and vasaline. All the dishes were placed in an incubator running at a constant temperature of 25° C. Spores were examined after 6 hours and 24 hours and the results obtained are given in Table XIV.

TABLE XIV

Germination of spores of C. sesami *at various relative humidities*

Gms. NaCl	Relative Humidity	6 hours		24 hours	
		% germination	Av. length of germ tubes (μ)	% germination	Av. length of germ tubes (μ)
0·0	100	45·5	24·2	89·6	69·5
4·5	97·3	29·6	15·8	66·0	42·2
7·0	95·7	16·6	11·5	41·2	31·4
10·0	93·4	8·4	8·0	18·1	19·9
12·0	92·7	5·9	4·7	12·4	11·5
15·0	91·0	..	..	..	..
16·0	90·0	..	..	..	..

It will be evident from Table XIV that the best germination was obtained at 100 per cent. relative humidity and it gradually decreased upto 91 per cent. when there was no germination.

(*d*) *Hydrogen-ion concentration.*—Spore germination is remarkably affected by hydrogen-ion concentration of the solution. Spore suspensions were made in solutions of known pH values and a drop mounted on a cover slip and inverted over Van Tiegham rings containing a few drops of the solution of the same pH values as the hanging drop. Adjusted modified Richards' solution of Karrer and Webb (1920) was used and the solutions of the following pH value were prepared : 2·1, 2·5, 2·7, 2·8, 3·3, 4·9, 5·9, 6·9, 7·3, 8·0 and 9·1. All the slides were incubated at 25° C. for 10 hours. The percentage of germination and the average length of the germ tubes were determined for each pH value. The results are recorded in Table XV.

TABLE XV

Germinatnation of spores of C. sesami *at different hydrogen-ion concentrations*

pH values	2·1–2·8	3·3	4·9	5·9	6·9	7·3	8·0	9·1
Percentage germination ..	..	51	70·0	97·0	24·5	11·5	6·0	..
Av. length of germ tubes (μ)	..	14	16·4	42·7	12·3	3·6	3·0	..

It will be evident from Table XV that the spores can tolerate a wide range of hydrogen-ion concentration from 3·3 to 8·0. The minimum hydrogen-ion concentration for germination lies between pH 2·8 and 3·3 and the maximum lies between 8·0 and 9·1 and the optimum is between 4·9 and 5·9.

VI. Hydrogen-ion Concentration

In order to determine the hydrogen-ion concentration relationship of the growth of *C. sesami* Richards' solution as modified by Karrer and Webb (1920) was used and his method was followed. Thirty c.c. of the solution together with the required amount of N/5 acid and N/5 alkali and distilled water to make 50 c.c. was put in each flask and hydrogen-ion concentration was determined according to the colorimetric method of Clark and Lubs (1917). Four flasks were prepared for each pH value ; one of these was put as control while the other three were inoculated. All the flasks were incubated for 56 days at 25° C. after which the dry weight of the mycelium of the fungus was determined. The hydrogen-ion concentrations of the controls and filtrate were also determined to see whether changes were brought about as a result of the metabolic activities of the fungus. The data obtained are given in Table XVI and represent the average dry weight of the mycelium from three flasks in each case.

Table XVI

Growth of C. sesami *at different pH and the changes in reaction induced*

Hydrogen-ion concentration			Average dry weight of the mycelium
Initial	Control after 56 days	Inoculated after 56 days	
2·1–2·7	2·1–2·7	2·1–2·7	No growth
3·3	3·3	2·6	0·1265
4·6	4·2	2·6	0·1621
5·5	4·9	2·6	0·1625
6·5	5·7	2·6	0·194
7·1	6·4	2·6	0·1901
8·1	6·4	6·6	0·0260
8·5	6·9	6·9	No growth

It will be noticed from Table XVI that the growth occurs over a range of pH varying from 3·3 to 8·1. Maximum growth occurs at 6·5 ; on the acid side no growth takes place beyond 3·3 and on the alkaline side beyond 8·1. The fungus during its growth produced marked changes in the reaction of the medium. It is seen from the table that there has been very little change in the pH values of the controls on the acid side of neutrality, while in those on the alkaline side there is remarkable shift in the pH values. The pH 2·1 to 2·7 remained constant after 56 days while pH 7·1, 8·1 and 8·5 shifted to

pH 6·4, 6·4 and 6·9 respectively. The pH 3·3, 4·6, 5·5, 6·5 and 7·1 of the inoculated flasks all shifted to a consatnt of 2·6 which therefore represents the acidity of the medium on which the fungus grew well.

VII. Summary

Cercospora sesami Zimm. is parasitic on leaves, petioles, stems and pods of *Sesamum indicum*. Symptoms of the disease have been described and the morphology of the parasite given in the text.

Inoculation experiments show that all the parts of *Sesamum indicum* are susceptible to the attack.

A study of the fungus was made on a large number of artificial media. Its growth is better on thickly poured plates than on thinly poured ones, more in alternate light and darkness than in complete darkness or continuous light.

The fungus can tolerate a wide range of relative humidity from 50 to 100 per cent., the optimum humidity for growth being 92 per cent.

The temperature relationship has been studied on Hopkins' and Richards' solution agar and the optimum has been found to lie between 25°—30° C.

On Coons' solution it has been found that the dry weight of the mycelium decreases with the decrease in concentration of the medium from the normal and increases with the increase in concentration above the normal.

Sugar is the most important constituent of Richards' solution influencing the growth of the fungus ; KNO_3, $MgSO_4$, KH_2PO_4 and $FeCl_3$ are next in importance in the order mentioned.

Media, alternate light and darkness and fluctuating temperatures are the most important factors influencing zonation in artificial culture.

Light, humidity, temperature, media and wounding have been found to influence sporulation in culture ; the details are given in the text.

The size and septation of the spores are influenced by temperature, humidity and media.

The effect of sugar, temperature, humidity and hydrogen-ion concentration on spore germination was studied and the details are described in the text.

The optimum hydrogen-ion concentration for the growth of the fungus is at 6·5. There is no growth at 2·1 to 2·7 and 8·5.

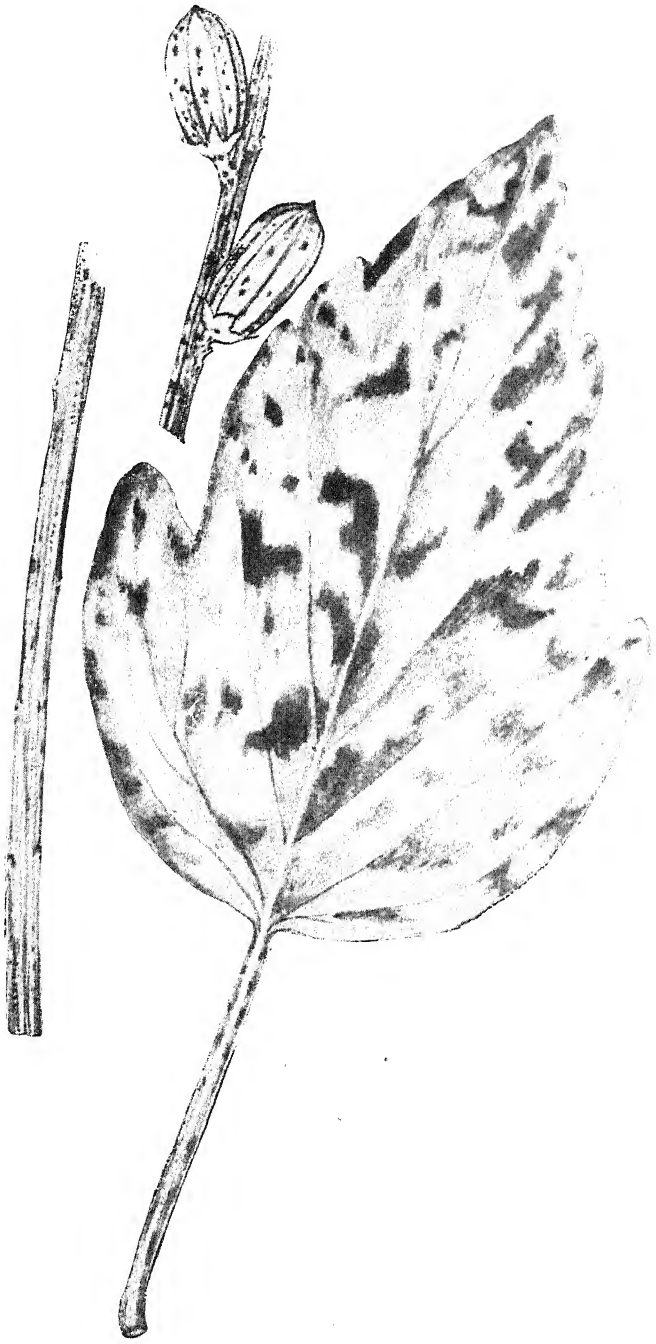


FIG. 1. *Cercospora sesami* on *Sesamum indicum*. Symptoms of the disease

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THE ENZYMES OF TWO WATER MOLDS

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Introduction

The enzymic reactions are absolutely necessary for any process associated with the cellular activity of a living organism. So far very little work seems to have been done with regard to enzymes of the members of the family Saprolegniaceæ. Of the various genera, *Achlya* sp. and *Saprolegnia Tokugawana* were studied by Emoto (1923); *Achlya bisexualis* and *Saprolegnia ferax* by Wolf (1937) and *Saprolegnia delica* by Saksena and Bhargava (1941). Very recently Bhargava (1943) studied the more important intra- and extra-cellular enzymes of *Achlya* sp., *Brevilegnia gracilis*, *Isoachlya* sp., *Saprolegnia delica* and *S. monoica* both qualitatively and quantitatively.

The present work deals with the estimation of more important extra- and intra-cellular enzymes present in *Achlya dubia* Coker and *Thraustotheca clavata* (deBary) Humph.

Material and Methods

Achlya dubia Coker was isolated from a local sample of water using ants as baits. Single spore cultures were prepared by the usual method. The culture of *Thraustotheca clavata* (deBary) Humph. was obtained from Centraal Bureau voor Schimmelcultures, Baarn, Holland. Throughout the investigation, the methods adopted by Bose and Sarkar (1937) and Bhargava (1943) were closely followed. For the details of experiments, the reader is referred to his paper (Bhargava, 1943). Since the fungi remain sterile in the medium used, determination of enzymes was made only at one stage, *i.e.*, of vegetative mycelium.

Experimental

Carbohydrases.—The presence of carbohydrases, *viz.*, amylase invertase, maltase, emulsin, cellulase and hemicellulase in the mycelium of the two organisms was tested qualitatively by their ability to reduce the solutions of the corresponding carbohydrates, such as, soluble starch, cane sugar, maltose and amygdalin, etc. To determine the amount of enzymic activity quantitatively, a reaction mixture was prepared together with a suitable buffer solution and the amount of reduced sugar was estimated by Shaffer and Hartman's method (1921). The mean results are given in Table 1.

TABLE I

Amount of reducing sugar (in mgs.) formed in 10 c.c. of the total digested volume out of 37 c. c. containing 0·1 gm. of fungus meal or 2 c.c. of extra-cellular enzymic solution

Enzyme	*Achlya dubia*		*Thraustotheca clavata*	
	Intra-cellular enzymic activity	Extra-cellular enzymic acitvity	Intra-cellular enzymic activity	Extra-cellular enzymic activity
Amylase ..	8·45	1·95	11·45	4·5
Invertase ..	5·25	0	8·65	0
Maltase ..	10·2	4·0	10·45	1·55
Emulsin ..	36·75	2·75	32·7	2·35
Cellulase ..	9·55	9·05	10·5	9·05
Hemicellulase ..	8·6	13·15	5·5	8·1

The results obtained in Table I indicate that generally the intra-cellular activity is much greater than the extra-cellular except in the case of hemicellulase. Invertase is absent as an extra-cellular enzyme in both the species.

Proteolytic Enzyme.—A suitable reaction mixture prepared with peptone, cirtate buffer and fungus meal or extra-cellular enzymate solution, was incubated in flasks for 24 hours at 37° C. and then 2 c.c. of the reaction mixture was titrated with 0·01 alcoholic KOH solution. The results obtained are gievn in Table II.

TABLE II

Amount of KOH solution (in c.c.) for 2 c.c. of the total digested volume of 12 c.c. containing 0·1 gm. fungus meal or 2 c.c. of the extra-cellular enzyme solution

Fungus	Intra-cellular enzymic activity		Extra-cellular enzymic activity	
	Control (inactivated)	Active enzyme	Control (inactivated)	Active enzyme
Achlya dubia	14·95	14·0	16·75	16·15
Thraustotheca clavata ..	14·95	14·5	16·75	14·5

The results obtained in Table II show the presence of proteolytic enzymic activity.

Lipase.—The presence of lipolytic enzyme was estimated by the method of Kanitz (1925). The reaction mixtures were titrated with N/25 KOH solution. The results are given in Table III.

TABLE III

Amount of 0·04 *N KOH* (*in c.c.*) *for the whole digested volume containing* 0·1 *gm. of fungus meal or* 2 *c.c. of extra-cellular enzyme solution*

Species	Intra-cellular enzymic activity		Extra-cellular enzymic activity	
	Control (inactivated)	Active enzyme	Control (inactivated)	Active enzyme
Achlya dubia	6·1	5·8	3·85	3·85
Thraustotheca clavata ..	6·2	5·9	2·95	2·95

From Table III it is seen that lipase is absent as an extra-cellular enzyme in both the species. There is, however, a slight intra-cellular activity.

Butyrase.—The flasks containing the suitable reaction mixtures were incubated at 40° C. for 5 days and were then titrated with N/50 KOH solution. The results are tabulated in Table IV.

TABLE IV

Amount of N/50 KOH (*in c.c.*) *solution required to neutralise the acid given out in hydrolisation in* 10 *c.c. of the reaction mixture*

Species	Intra-cellular enzymic activity		Extra-cellular enzymic activity	
	Control (inactivated)	Active enzyme	Control (inactivated)	Active enzyme
Achlya dubia	7·65	5·2	5·2	4·8
Thraustotheca clavata ..	7·65	5·0	5·2	4·7

The above results show that butyrase is present both as an extra- and intra-cellular enzyme.

Catalase.—The suitable reaction mixture was incubated at room temperature for two hours, after which it was titrated with 0·1 N $KMnO_4$ in presence of sulphuric acid. The results obtained are given in Table V.

TABLE V

Amount of 0·1 *N* $KMnO_4$ (*in c.c.*) *required for* 5 *c.c. of hydrogen peroxide solution containing* 0·1 *gm. fungus meal or* 2 *c.c. extra-cellular enzyme solution*

Species	Control (inactivated)	Intra-cellular enzymic activity	Extra-cellular enzymic activity
Achlya dubia	2·0	1·0	2·0
Thraustotheca cavaata ..	2·0	1·5	2·0

It is seen that the enzyme shows no extra-cellular activity, though it is present as an intra-cellular enzyme.

Laccase, Tyrosinase, Rennetase and Oxidase.—Various tests were made to test the presence of laccase, tyrosinase, rennetase and oxidase. The results obtained indicate that the above mentioned enzymes are altogether absent in the two species studied by the authors.

Discussion

The function of carbohydrases is to convert more complex carbohydrates into such simpler compounds as can be used directly by the organism. The presence of a particular enzyme in an organism indicates that the organism is able to utilise the corresponding carbohydrate. From the results obtained in Table I, it is evident that the fungi under investigation, *viz.*, *Achlya dubia* and *Thraustotheca clavata* are able to secrete all the carbohydrases tested, except invertase. This shows that sucrose will not be assimilated by them. The results obtained in the present case are in entire agreement with those obtained by Bhargava (1943) for some of the water molds used by him. The present organisms too, like other watermolds, differ from *Brevilegnia gracilis*, a parasite, but a member of the family Saprolegniaceæ.

Like the results obtained by Emoto (1923) and Bhargava (1943), proteolytic activity, though feeble, is present in *Achlya dubia* and *Thraustotheca clavata*.

As regards lipase, Emoto (1923) reported its absence in *Achlya* sp. and *Saprolegnia Tokugawana*; Wolf (1937) found it to be present in *Achlya bisexualis* and *Saprolegnia ferax*; Saksena and Bhargava (1941) pointed out that it was not secreted by *Saprolegnia delica* and Bhargava (1943) also obtained similar results with other fungi. In the present instance also it is not secreted by the fungi investigated.

Butyrase is present both as an intra- and extra-cellular enzyme. The results obtained by Bhargava (1943) show that it was present as intra-cellular enzyme only in the case of water molds studied by him.

Catalase has been found to be present as an endoenzyme only.

The present authors also, like Bhargava (1943, p. 97), are inclined to the view that the activation of the atmospheric oxygen into a more chemically reactive state is not required by the water molds. Therefore oxidase, laccase, tyrosinase and rennatase which are oxidising enzymes, are not produced in the species under investigation.

Summary

Some of the more important enzymes of *Achlya dubia* Coker and *Thraustotheca clavata* (deBary) Humph. have been estimated.

Among the carbohydrases amylase, maltase, emulsin, cellulase and hemicellulase were present both as extra-cellular and intra-cellular enzyme. Invertase was present as intra-cellular enzyme only. In all cases except the hemicellulase the amount of intra-cellular enzyme was greater than the corresponding extra-cellular one.

Proteolytic enzyme was present in small quantities. Lipase and catalase showed only a slight intracellular activity, while butyrase was present both as extra- and intra-cellular enzymes.

Laccase, tyrosinase, rennetase and oxidase were found to be absent.

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THE VEGETATION OF THE RAJGHAT RAVINES

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I. Situation, Topography and Development of the Ravines

A large number of ravines bearing forest growth dissect the sides of the plateau of Rajghat as it stands to the north-east of Benares town separated from it by a depression carrying the Grand Trunk Road and Kashi station of the East Indian Railway situated on the ridge. The plateau itself is a rectangular area about $\frac{3}{4}$ mile long and $\frac{1}{4}$ mile broad with the Ganges scoring on the south and the river Barna circumscribing it on the north and the east where it meets the former river (Fig. 1). It is about 255 feet above the sea and the rivers run 55 feet below during the dry season. The exposed banks of the rivers are then cultivated.

The ravines are formed on account of gully erosion. The monsoon bringing the rain releases it generally in a torrential downpour during the rainy season. The water collecting and running on the periodically rain battered ground of the plateau scores out small gullies. As the various ramifications of these unite and the intensity of soil erosion increases with the increasing volume of running water the ravines are dug deeper and wider at lower levels when approaching the rivers. Occasional landslides cut the sides precipitously giving them the appearance of deep gorges. The top of the plateau slopes gradually from the south to the north and hence the ravines are longer towards the Barna than those running into the Ganges.

II. History and Biotic Factors

Although the development of the ravines is so rapidly ruinous to the plateau yet it has a marvellously long history of existence though undoubtedly getting spatially narrowed on the north and the south. The area was once thickly inhabited as is clear from the traces of old buildings, the Rajghat Fort and from the more recent excavations conducted by the department of Archæology. It is now the property of the Rishi Valley Trust who have built on the site a residential school and college. The buildings including the old temple of Adikeshwar extend up to the confluence of the rivers.

The stability of the plateau is chiefly dependent on the interesting vegetation of the ravines which has appreciably slowed down the progress of erosion if it has not already arrested it at certain places. Wherever the growth of this vegetation has been interfered with it

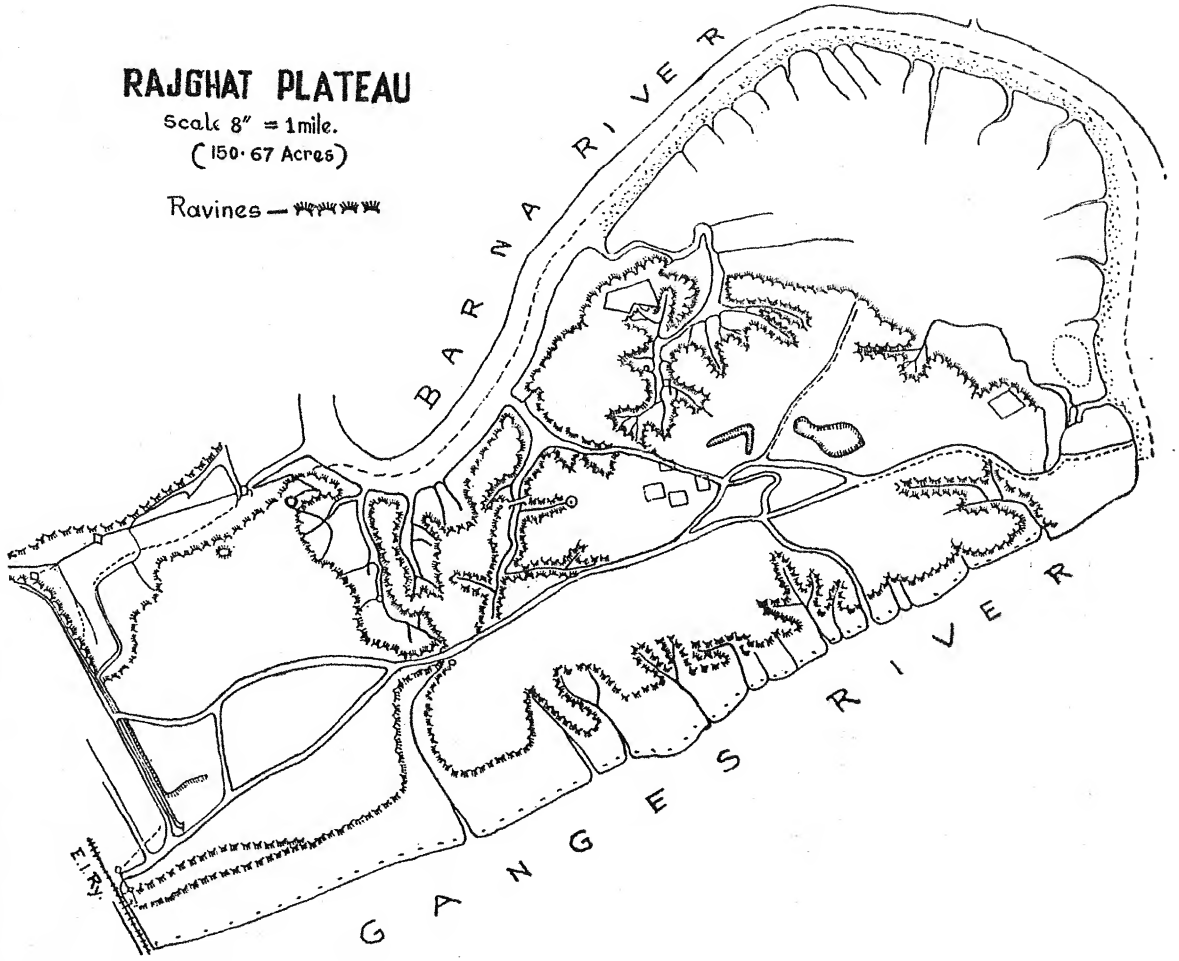

RAJGHAT PLATEAU
Scale 8" = 1 mile.
(150·67 Acres)
Ravines
BARNA RIVER
GANGES RIVER
E.I.Rly.

FIG. 1

has led to ruin and misery. The clearing of the vegetation for the archæological excavations on the south-west corner of the plateau and the college buildings on the north-eastern ridge has already made their position precarious. Further, the ravines contain abundant remnants of buildings including a huge stone gate of the old fort which has collapsed on the Barna side. These point to the havoc wrought by erosion due to the destruction of the vegetation subject to periodic pressure of human population on the historical plateau.

Clearing of the vegetation especially about a ridge or a rudimentary ravine for building purposes is therefore a process ruinous to the vegetation and the plateau. Felling the trees for timber and fuel and lopping are common practice and continuous grazing of cattle, sheep and goat limit the growth of the forest in the ravines. Scraping of the ground vegetation which may at times cover parts of the plateau exposes the loosened soil for rapid erosion. Most of the herbs including the taller rainy season annuals dry up in the cold season when they are collected for fuel and not allowed to decay on the soil which might improve its fertility.

III. Climate*

The periodic climate of Benares is typical of the Upper Gangetic Plain with an annual average rainfall of 40 inches. The rainy season extends from about the middle of June to September when nearly 36 inches of the fall are obtained ; July and August being the wettest months. The average number of rainy days in the season is 20 per month, and the mean relative humidity is about 88%. The mean maximum temperature during the period is about 90·5° F. and the corresponding minimum is 78° F. This is the most favourable season for plant growth as it follows the dry hot summer.

The bright month of October is rainless and calm. The mean maximum temperature is 94·7° F. but the mean minimum is 65·6° F. on account of the cooler nights. This is followed by the cold season of 4 months when the days are cool and bright with a breeze from the west. The nights are cold and humid. There may be a little rainfall late in the season but it seldom exceeds 2 inches in any month. The mean maximum and minimum temperatures for the season are 80·8° and 52·8° F. and the mean relative humidity is 79·9%.

The month of March is a mild warm period of transition between the cold season and the following hot season ; the latter extends from April to the middle of June. The mean maximum temperature during the hot season is about 105° F. but the absolute maximum temperature may go upto 115° F. in May. Dry hot wind known as " loo " blows strongly from the west during the day time. It desiccates plant tissues very often killing them. But the narrower ravines are not much exposed and as the wind sweeps over the rivers before turning into them the protected vegetation does not suffer much on this

* The figures given in this section are based on records for 1942-43 (Misra, 1944).

account. The nights are not so hot and the mean minimum temperature for the season is 76·6° F. The mean relative humidity for the period is about 48%. The weather becomes stormy at times with occasional drizzles following dust. However, erosion of soil by wind is moderate.

The ravines in the south to the Ganges side receive more of sun than those in the north towards the Barna. This factor seems to be responsible for differences in the floristic details of the plant communities present in the two systems.

IV. Soil

The plateau consists of a fairly uniform deposit of an old alluvium on a bed of 'kankar'; the latter is found locally exposed on the bank of the Barna. The soil is light being moderately porous and gray and brown coloured with small rounded grits and calcareous nodules. It is alkaline with pH values ranging between 7·90 and 8·34 and it gives a strong positive reaction for nitrates when tested with a 0·02% solution of di-phenyl-amine in concentrated sulphuric acid (Misra, 1944). The ravines at their lower approaches to the river get covered with newer sandy deposits every year on inundation when it is in spate during the latter part of the rainy season. The coarser soil is pale coloured and generally richer in carbonates.

V. Vegetation

1. *General characters of the vegetation*

The pioneer species which consolidates the soil against erosion in the area is *Capparis sepiaria*. On account of the thorns on the plant it also affords protection to the vegetation growing under it against grazing. As a result *Diospyros cordifolia* followed by a number of deciduous trees rapidly grow up to form a closed forest. Most of these and especially the two species named above are capable of regenerating from buds developing on their roots as they get exposed on erosion of the soil. These can therefore establish along with some annuals on the precipice also. Thus the ravines get completely covered with a thick mantle of forest which becomes quite impenetrable by the middle of the rainy season every year so effectively checking soil erosion indeed, at a time when its absence might be disastrous to the plateau.

With the dying of the annuals, slower growth of the perennials and indiscriminate grazing and removal of wood for fire in the following cold season the forest becomes thinner. The uppermost storey consisting of the deciduous trees opens up still further by the fall of the leaves in the beginning of the hot season. The forests of the ravines become poorer with the march of the dry seasons since the intensity of the biotic factors increases simultaneously with a gradual decline in the growth of the vegetation. Continued grazing and scraping of the ground vegetation prepare the plateau for a fresh assault by the monsoon erosion which has been shown to be severe in the earlier part of the rainy season.

2. *Plant communities*

The following plant communities have been recognised in this study. The species marked with an asterisk are either exclusively confined or more abundant in the sunny Ganges ravines. The notations used are:—*d*–dominant, *cd*–codominant, *a*–abundant, *f*–frequent, *o*–occasional, *r*–rare ; *v* and *l* used as prefix signify 'very' and 'local' :—

1. *Holoptelia—Albizia—Cordia* association

Holoptelia integrifolia, Planch.	*d*	*Feronia elephantum*, Correa.	*r*
Albizia lebbek, Benth.	*cd*	*Tamarindus indica*, L.	*r*
Cordia myxa, L.	*cd*	*Ficus religiosa*, L.	*r*
Pongamia glabra, Vent.	*o-f*	*Ehretia acuminata*, Br.	*vr*
Melia Azadirachta, L.	*o*	*Mitragyna parvifolia*, Korth.	*vr*
Acacia leucophlaea, Willd.	*o*		

2. Consociations of *Holoptelia integrifolia*, *Albizia lebbek* and *Cordia myxa*

3. Societies of *Pongamia glabra* and *Acacia leucophlœa*

4. *Capparis—Diospyros* associes

Capparis sepiaria, L.	*d*	**Lantana indica*, Roxb.	*f*
Diospyros cordifolia, Roxb.	*cd*	*Cocculus villosus*, DC.	*r*
**Capparis horrida*, L.	*o*	*Coccinia indica*, W. and A.	*o*
**Clerodendron phlomidis*, L.	*o*	*Quamoclit pinnata*, Boj.	*o*
**Abrus precatorius*, L.	*o*	*Rhynchosia minima*, DC.	*o*
Abutilon indicum, G. Don.	*o*	*Cardiospermum halicacabum*, L	*r*
A. graveolens, W. and A.	*o*	*Melothria heterophylla*. Cogn.	

The climbers become more abundant during the rainy season.

5. Consocies of *Capparis sepiaria* and *Diospyros cordifolia*

6. Associes of *Ficus glomerata—Pongamia glabra*

Ficus glomerata, Roxb.	*d*	*Ficus religiosa*, L.	*o*
Pongamia glabra, Vent.	*cd*	**Dalbergia sissoo*, Roxb.	*r*

7. *Saccharum* consocies

Saccharum munja, Roxb.	*d*	*Desmostachya bipinnata*, Stapf.	*f-a*
S. Ravennae, L.	*f*	*Indigofera tinctoria*, L.	*o*
Alhagi camelorum, Fisch.	*a*		

8. A scrub colony

Acacia arabica, Willd.	*f*	*Phoenix sylvestris*, Roxb.	*f*
Zizyphus jujuba, Lamk.	*f*	*Streblus asper*, Lour.	*o*

9. Colonies and societies of herbs

(*a*) On precipice :

Aerua scandens, Wall.	*Linaria ramosissima*, Wall.
Aristida adscensionis, L.	*Lindenbergia urticaefolia*, Lehm.
Cenchrus ciliaris, L.	*Peristrophe bicalyculata*, Nees.
Chloris virgata, Sw.	*Pulicaria crispa*, Benth.
Digitaria sanguinalis, Scop.	

(*b*) On moderately eroded land :

Abutilon indicum, G. Don.
Aerua scandens, Wall.
Blepharis boerhaaviaefolia, Pers.
B. molluginifolia, Pers.
Desmostachya bipinnata, Stapf.
Dichanthium annulatum, Stapf.
Indigofera tinctoria, L.
Nepeta ruderalis, Ham.
Pulicaria crispa, Benth.
Pupalea lappacea, Moq.
Saccharum munja, Roxb.

(*c*) On open grounds :

Achyranthes aspera, L.
Blumea spp.
Boerhaavia diffusa, L.
†*Cassia occidentalis*, L.
†*C. tora*, L.
†*Crotalaria medicaginea*, Lamk.
Cynodon dactylon, Pers.
Cyperus rotundus, L.
Dichanthium annulatum, Stapf.
Digera arvensis, Forsk.
†*Digitaria sanguinalis*, Scop.
Euphorbia hirta, L.
E. prostrata, Ait.
E. thymifolia, L.
Indigofera ennaephylla, L.
Jatropha gossypifolia, L.
Justicia diffusa, Willd.
Ocimum canum, Sims.
Sida rhombifolia, L.
†*Urochloa reptans*, Stapf.
Vernonia cineria, Less.

(*d*) On shaded grounds :

Acalypha ciliata, Forsk.
A. indica, L.
Achyranthes aspera, L.
**Anisomeles ovata*, R.
**Barleria prionitis*, L.
Biophytum sensitivum, D. C.
Boerhaavia repanda, Willd.
†*Commelina benghalensis*, L.
†*Corchorus acutangularis*, Lam.
Desmodium gangeticum, DC.
Eleusine aegyptica, Desf.
Eragrostis tenella, Stapf.
Hyptis sauveolens, Poit.
Malvastrum tricuspidatum, A. Gray.
‡*Nepeta ruderalis*, Ham.
‡*Nicotiana tabacum*, L.
†*Oplismenus Burmanii*, Beauv.
Oxalis corniculata, L.
†*Paspalidium flavidum*, Stapf.
†*Peristrophe bicalyculata*, Nees.
Phyllanthus niruri, L.
†*Physalis minima*, L.
Ruellia prostrata, Poir.
R. tuberosa, L.
Rungia parviflora, Nees.
‡*Salvia plebefa*, Br.
†*Setaria intermedia*, Roem. and Sch.
†*S. plicata*, T. Cooke.
†*S. rhachitricha*, T. Cooke.
Sida veronicaefolia, Lamk.
Solanum nigrum, L.
S. verbascifolium, L.
†*Triumfetta neglecta*, W. and A.
Vernonia cinerea, Less.

The species marked with † are found in the rainy season and those with ‡ come up in the cold season only.

(*e*) On annual deposits laid down by the river :

Alhagi camelorum, Fisch.
Alternanthera sessilis, Br.
A. paronychoides, Fort.
Argemone mexicana, L.
Celsia coromandelina, Wall.
**Chrozophora Rottleri*, A. Juss.
Cochlearia flava, Ham.
Croton sparsiflorus, Morung.
Cynodon dactylon, Pers.
**Datura alba*, Nees.
Euphorbia spp.
Gnaphalium spp.
Grangea maderaspatana, Poir.
**Heliotropium ovalifolium*, Forsk.
**Lantana indica*, Roxb.
Lippia nodiflora, Rich.
Mollugo hirta, Thunb.
Polygonum plebejum, Br.
Potentilla supina, L.
Rumex dentatus, L.
Veronica anagallis, L.
Xanthium strumarium, L.

3. *Structure and distribution of the communities*

The much eroded and grazed upper ravines bear the *Capparis-Diospyros* associes. The dominant species of the associes are evergreen thorny shrubs which grow upto a height of 10 to 20 feet covered with leaves from the base to the top and are usually infested with a large number of climbers. The community becomes closed in the narrower ravines and the gaps elsewhere are filled up by the ground vegetation which grows very dense during the rainy season when the taller herbs become continuous with the lower shoots of the dominant species. The plateau itself is locally covered by the consocies of *Capparis sepiaria* which, however, is too open due to the intensity of the biotic factors.

The forest association of *Holoptelia-Albizia-Cordia* comes up in the ravines where the *Diospyros-Capparis* associes has minimised grazing and erosion of soil. The dominant trees attain a height of 40 to 60 feet forming a closed canopy at the top which is penetrated by light only in April and May when the leaves are shed off. The second storey of this association consists of young trees and the associes and the consocies of *Capparis* and *Diospyros* on low ridges as these run under the forest canopy. The ridges originally separated the young ravines which have now fused on growing bigger and the neighbouring trees have overtopped them. The lower ravines which get inundated by the river during the rainy season have developed the *Ficus-Pongamia* associes and here societies of *Pongamia glabra* and *Acacia leucophlœa* generally line the association on the Barna side. Where the association is open and sand has been deposited at the bottom of the ravines fragments of a scrub colony of *Acacia arabica*, *Zizyphus jujuba* and *Phœnix sylvestris* are often met with. They generally follow a consocies of *Saccharum*. These communities would undoubtedly show a better development on the sloping banks of the rivers but for the intense cultivation.

The development of the association is completely arrested at the top of the plateau on account of the biotic factors. There is a small and very open relict consociation of *Holoptelia integrifolia* in the north-east ; the rest of the area is sprinkled with the consocies of *Capparis*.

The herbaceous layer of the association is composed of elements characteristic of situations exposed to erosion, sun and shade and those of the sandy river banks. They show marked seasonal aspects and have already been listed as forming colonies and societies.

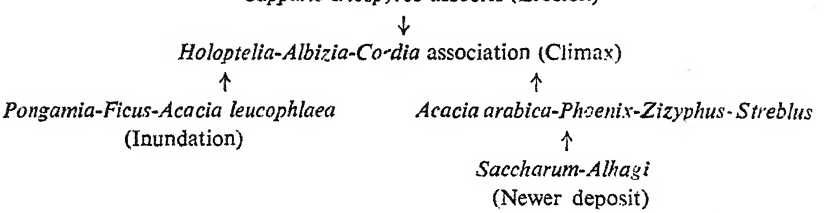
It will be seen that the stratification of the ravine forest association is chiefly on account of a corresponding layering of the soil. The tree layer is supported by the deeper old alluvium, the shrub layer of *Capparis-Diospyros* associes runs underneath on rough lands subjected to monsoon erosion and the ground vegetation is dependent chiefly on the surface soil. *Capparis sepiaria* snd *Diospyros cordifolia* are indifferent to light conditions and since the community maintains its identity with the factor of erosion even under the tree layer it has

been regarded as an independent associes though by structure it forms here only a layer society of the association. Similarly the ground vegetation on the sand, in the lower ravines, has not much to do with the top layers. However, the societies and the colonies dependent on the shade have to be regarded as parts of the association.

The unstable precipice bears patchy colonies of herbs. This is gradually succeeded by the *Capparis-Diospyros* associes which hangs down from the ridge and the species root on the vertical earth. A landslide may rarely cut across the tree covered areas. On the Barna side *Pongamia glabra* and on the sunny Ganges side *Dalbergia sissoo* regenerate more quickly than the other species can do from buds developing on the exposed roots attached to the precipice.

4. *Successional relationships*

The plant communities are developmentally related as shown below :—



VI. Discussion

The vegetation of the Rajghat ravines is nearer the climax of the Upper Gangetic Plain which has been described as a monsoon deciduous forest by Dudgeon (1920) and a tropical dry deciduous forest by Champion (1936). In this respect it is very different from the thorn-scrub type of vegetation consisting of species of *Acacia* or, on saline soils, *Butea frondosa* as is generally obtained in the ravines round about Benares. Erosion of soil and its low capacity for retaining moisture on account of rapid run off and poor plant cover are chiefly responsible for the existence of the thorn-scrub in such areas. But the Rajghat-ravines running down from the plateau and surrounded by the two perpetual rivers actually enjoy better humid conditions and here the old fertile alluvium is more retentive of moisture in spite of the rapid run off. The communities of *Capparis-Diospyros*, *Saccharum-Alhagi* and *Acacia-Phœnix-Zizyphus* have been shown to be largely dependent on the surface soils and these afford the necessary protection against grazing and thus lead to the development of the deciduous forest association which is sustained by the deeper old alluvium. This association is not large enough here to include many of the constituent species of the climax type as detailed by Champion (1936) for certain places in this province. But it seems to have a relict value fairly indicating that *Holoptelia integrifolia*, *Albizia lebbek* and *Cordia myxa* are the dominant species of the type for this area.

VII. Summary

The development of the ravines and their vegetation have been described in relation to the environmental factors as obtained at the plateau of Rajghat.

Altogether nine types of plant communities have been recognised. Their structure, distribution and successional relationships are detailed and the nature of the climax vegetation is discussed.

VIII. Acknowledgment

The author is thankful to Prof. Y. Bharadwaja for facilitating the work and to Principal N. S. Rama Rao of Besant College, Rajghat, for lending him the map of the plateau.

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A NEW *ARTABOTRYS* FROM BURMA

By D. Chatterjee

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The circumstances which led to the discovery of this new plant are interesting. It was first collected by Parkinson in 1929 from tropical rain forests in the Bassein District of Lower Burma. From its resemblance to the common *Artabotrys odoratissimus* R.Br. it was at first thought to be the same. Later it was sent to the Dehra Dun herbarium where it was again identified as *A. odoratissimus.* It was lying under this name in the Forest Herbarium, Maymyo, till 1942 when in the course of a revision of the Burmese Anonaceæ undertaken at Mandalay by the author it was recognised as differing considerably from *A. odoratissimus.* The sheets, together with some others of doubtful determination, were sent to Calcutta by the author shortly before the evacuation of Burma. Later the author was able to have them compared with the Wallichian sheets of *A. odoratissimus* by the kindness of Dr. S. K. Mukherjee, who remarked that "Parkinson's No. 8747 and No. 5060 are very different from Wallich's No. 6418 and these differ amongst themselves and are two distinct species." Parkinson's sheets No. 8747 were sent to Kew for comparison and were returned as "*Artabotrys* not matched; not *A. odoratissimus* R.Br." Parkinson's No. 8747 is described below. The material of his No. 5060, though sufficient to establish its distinctness, is too scanty for a full description.

Artabotrys Parkinsonii Chatterjee *Sp. Nov.* (Anonaceæ).

Planta distinctissima, Artabotrys odoratissimo habitu foliisque similis, sed ab eo pedunculi floribus minus numerosis minoribusque, petalis obtusis, minutissime pubescentibus, fructu anguste elongato, apice mucronato, inter alia satis recedit.

Extensive climber. Stem and branches terete, brown, longitudinally and minutely wrinkled at least when dry, glabrous, thinly lenticellate. *Leaves* shortly petioled, simple, alternate, lanceolate or elliptic-lanceolate, entire, acute, base cuneate, chartaceous, both surfaces glabrous, upper surface shining; main nerves thin and rather inconspicuous, 7–9 pairs, spreading and anastomosing in loops by their ends below the margin; secondary nerves irregularly transverse. Lamina 9–12 cm. long and 3–3.5 cm. wide; petiole short, 3–5 mm., darker than the midrib, shallowly channelled. *Peduncles* usually leaf-opposed, hook flattened, curved, with about 6–8 flowers on each hook (2–3 flowers at the end of first curvature and some 4–5 flowers at the far end); hooks glabrous except near the bases of pedicels which are minutely rufous-tomentose. *Bracts* 2, minute, subulate, rufous-tomentose. *Flowers* regular, bisexual, greenish, 2–2.5 cm. in diam. Pedicel thinly hairy and gradually thickened upwards near the bases of sepals, 1–1·5 cm.

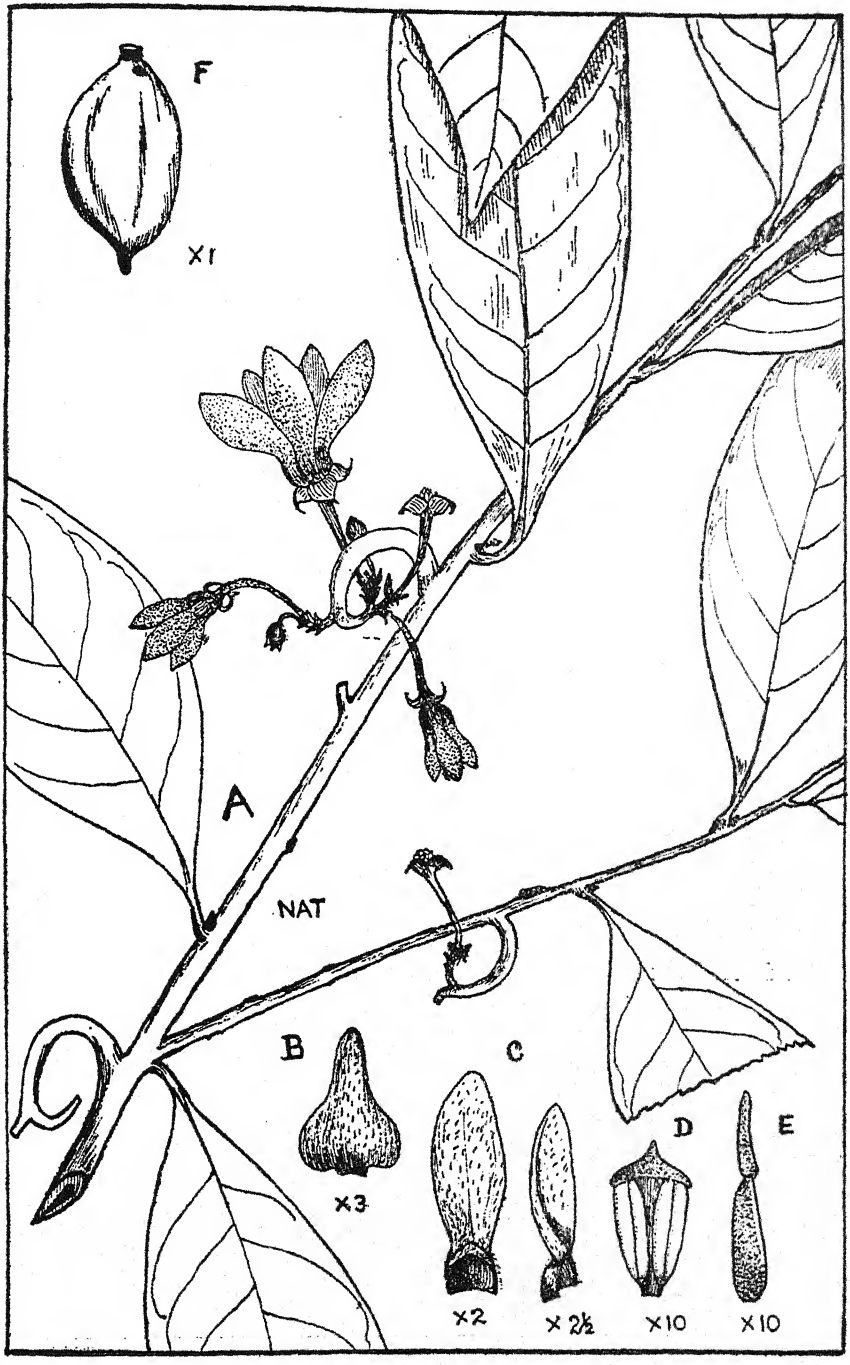

Fig. 1. *Artabotrys Parkinsonii* Chatterjee *Sp. Nov.*

long. *Sepals* 3, valvate, broadly deltoid, shortly acuminate, coriaceous, 4–5 mm. wide, hairy on both surfaces with golden brown hairs. *Petals* 6, biseriate, free; outer series larger, coriaceous, consrticted near the base, upper part broadly ovate-lanceolate with obtuse apex, both surfaces thinly hairy except the dark and glabrous inner side of the shallow concavity near the base below the constriction, 1.5 cm. long and .5 cm. wide; a thin, minutely and densely tomentose strip is present just above the glabrous area of the petal below the constriction; inner petals slightly smaller in size, alternating with the outer, constricted near the base like the outer petals but the concavity at the base is very deep and the inner petal looks like a cochleate lamina; the concavity is dark and glabrous and the limb is thinly hairy; 1 cm. long and .3 cm. wide. *Stamens* many, free, sessile, connective produced to form a cushion-like top with mucronate apex at the centre 2 mm. long, anther-lobes dorsal and narrowly elongated. *Carp ls* many, free, ovary narrowly conical, glabrous; stigma cylindrical, smooth, slightly and gently curved, glabrous, fluted on the top of the ovary where there is a constriction; about 2.5 mm. long with stigma. *Fruit* of 10–12 ripe carpels at the end of the peduncle which is swollen and much thickened; ripe carpels ellipsoid, dry, subsessile, indehiscent glabrous, with hard mucronate tip. Pericarp rather fibrous, 3 cm. long, 1.7 cm. wide. *Seed* one in each carpel, with ruminate endosperm.

Burma—Bassein District; Pyinmadon Chaung, *C.E. Parkinson* No. 8747, dated the 15th February 1929 (type and cotype in Herb. Calcutta).

This plant resembles *Artabotrys odoratissimus* in general habit and foliage, but differs in having more flowers on each peduncle, smaller size of the flowers, minutely pubescent obtuse petals, and slightly longer fruit with mucronate hard apex.

The work was carried out partly at the Agricultural College, Mandalay, and partly at the Botanical Laboratories of the Cotton College, Gauhati, Assam. The author acknowledges with grateful thanks very valuable help and encouragements received during the investigation from Dr. N. L. Bor, Mr. D. Dhind, Dr. K. Biswas, Dr. S. K. Mukerjee and Mr. V. Narayanaswami.

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ON REDUCTION DIVISION AND AUXOSPORE-FORMATION IN *CYCLOTELLA MENEGHINIANA* KÜTZ.*

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INTRODUCTION[1]

IT is now fairly well established through the excellent investigations of Klebahn (1896), Karsten (1896, 1897*a*, 1897*b*, 1899, 1900 and 1912), Geitler (1927*a*, 1927*b*, 1928 and 1932), Cholnoky (1927, 1928, 1929 and 1933*a*) and Meyer (1929) that auxospore-formation in the Pennales is the result of a sexual process. In the Centrales, on the other hand,

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[1] A preliminary note of this paper was published in *The Journal of the Indian Botanical Society*, 1942, Vol. XXI, Nos. 3 and 4, pp. 231–37.

auxospore-formation is considered to be an asexual or vegetative process and not a sexual one (Oltmanns, 1922 ; Karsten, 1928 : Hustedt, 1930 ; Smith, 1933 ; and Fritsch, 1935). Again, it has been fairly well established that the vegetative phase in the Pennales is diploid, reduction division taking place during auxospore-formation (*loc. cit.*, Klebahn, Karsten, Geitler, Cholnoky, Meyer, Smith and Fritsch), whereas in the case of the Centrales the vegetative phase is generally held to be haploid (Oltmanns, 1922 ; Hustedt, 1930). But some of the recent investigations on the group (Persidsky, 1929, 1935 and Cholnoky, 1933*b*) tend to point out that auxospore-formation in the Centrales also is brought about by a sexual process as in the Pennales and that the vegetative phase even in the Centrales is diploid as in the Pennales, reduction division taking place during auxospore-formation. The number of investigations, however, are so few and the details so meagre that algologists feel that the case for the Centrales is not quite fully established. Fritsch (1935, p. 620) commenting on the observations of Persidsky (1929) on *Chætoceros* and of Cholnoky (1933*b*) on *Melosira arenaria* states : " The possibility of a reduction division and of subsequent autogamy cannot be denied, but further research will be necessary to substantiate this clearly." Geitler (1935, p. 160) states : " Sexual reproduction among the Centrales, with the exception of *Melosira*, is not fully understood. It is probable, however, that the Centrales are also diplonts." Smith (1938, p. 213) when dealing with the Centrales refers to the recent investigations (*viz.*, Persidsky, 1929, 1935 and Cholnoky, 1933*b*) and finally states : " The nuclear behaviour in the foregoing cases is not established beyond all doubt, but there is a presumption that auxospore-formation is sexual in nature since it involves a fusion of two haploid nuclei. There is also a possibility that auxospores of other Centrales are formed in a similar manner. If this be true, vegetative cells of Centrales are diploid instead of haploid."

The centric diatom *Cyclotella Meneghiniana* Kütz., occurred in plenty at Madras. Advantage was taken of its profuse growth to study its life-history with special reference to the nuclear changes taking place during auxospore-formation.

Material and Methods

Occurrence

The diatom, *Cyclotella Meneghiniana*, was growing in two places at Madras : (1) in a temple tank at Triplicane and (2) in an artificial tank in a garden at Mount Road. It was growing along with various other planktonic algæ such as *Chlamydomonas*, *Scenedesmus*, *Pediastrum*, *Euglena*, *Phacus*, etc., and occurred in plenty from July to October 1940.

Cultures

The diatom was grown in the laboratory in liquid as well as in agar cultures for studying its life-history and cytology.

Liquid cultures.—The method followed by Gross (1937) was used with slight modifications for culturing the present diatom. All the

glass vessels used for the cultures were thoroughly cleaned with a mixture of sulphuric acid and bichromate of potash, washed thoroughly in tap water, then in distilled water and finally dried in a hot air oven (Rawlins, 1933). Petri-dishes were used for the cultures, since the cultures could be easily examined directly under the microscope frequently. Only glass-distilled water uncontaminated by contact with any metal was used for the cultures.

Bacteria-free cultures of the diatom were not aimed at, since the presence of the bacteria in the cultures did not appear to seriously affect the growth of the diatoms. In order to obtain a culture of the diatom as far as possible free from other algæ the following method was adopted. To start with, rough cultures of the diatom were made by picking out the individuals with the aid of a narrow pipette under a Greennough dissecting microscope and transferring them into the culture solution. From the preliminary rough cultures, the diatom cells were again picked out as before with the aid of a narrow pipette under the dissecting microscope and transferred to petri-dishes containing sterilized pond water. From these vessels the diatom cells were again picked out in the same manner and transferred into other petri-dishes containing fresh sterilized water. This process was repeated several times until a fairly pure culture of the diatom was obtained. Finally from this culture the diatom was picked out and transferred to sterilized culture solutions and left growing in them.

For cultures the following two solutions were used : (1) ' Erdschreiber' solution of Föyn (1934) and (2) Allen's (1910) modification of Miquel's solution further modified by Ketchum and Redfield (1938) as per the following formulæ :

(1) ' *Erdschreiber* ' *solution*

Sodium nitrate	0·1 gm.
Sodium acid phosphate	0·02 gm.
Soil decoction	50 c.c.
Filtered and sterilized pond water	1000 c.c.

(2) *Miquel's solution*

Solution A—

Potassium nitrate	20·2 gm.
Distilled water	100 c.c.

Solution B—

Sodium acid phosphate	4 gm.
Calcium chloride	4 gm.
Ferric chloride	2 c.c.
Conc. hydrochloric acid	2 c.c.

Diluted to 100 c.c. with distilled water.

To each litre of sterilized pond water, 0·55 c.c. of solution A and 0·5 c.c. of solution B was added.

For preparing the culture solutions the pond water in which the alga was growing was used. This water was filtered through a porous candle and then sterilized in an autoclave at 120° C. for 20 minutes.

The soil decoction for the 'Erdschreiber' solution was prepared by adding one kilogram of fine garden soil to one litre of distilled water and heating it in a steam-bath for nearly two hours. On the next day the supernatant liquid was separated and filtered into a flask and then sterilized.

The growth of the diatom in 'Erdschreiber' solution is very slow to begin with. But the diatom continues to grow in it for several months. In Miquel's solution, on the other hand, the diatom multiplies very rapidly in the beginning, but, if kept in the same medium for a long time, begins to degenerate and die. Even, if reinoculated into fresh solution, it does not show any signs of growth, but ultimately dies. On the other hand, if the diatom, when showing signs of degeneration in the Miquel's solution, is transferred into 'Erdschreiber' solution, it begins to thrive well again and keeps growing in a healthy condition for several months. The following procedure was, therefore, adopted throughout the investigation. The diatom was first grown in Miquel's solution for about 20 days for securing plenty of growth and then transferred into 'Erdschreiber' solution where it continued to remain in a healthy condition, though growing very slowly.

Agar cultures.—Diatom cells from fairly pure liquid cultures were picked out and pipetted into slightly warm 2% 'Erdschreiber' agar in test-tubes and, after shaking, dilution cultures were made. The brownish dot-like colonies of the diatom which are formed in these cultures are removed and streaked into fresh agar plates. The diatom multiplies fairly well in these plates and often assumes a filamentous condition (*cf.* below).

For cytological studies material from the cultures was fixed during the twenty-four hours of the day at intervals of one hour each. Material freshly collected from the field was also fixed in the same manner. The most abundant cytological stages were obtained in material fixed between 5 A.M. and 9 A.M.

The following fixing fluids were used :—Fleming's weak formula, Schaudinn's sublimate-acetic-alcohol (acetic acid 5%), Allen's modification of Bouin's fluid ($P.F.A_3$). Of these hot Schaudinn's solution and $P.F.A_3$ gave the best results. The material in both cases was left in the fluid for 5 to 12 hours and then washed, the washing being done with the aid of a centrifuge. The Schaudinn material was washed several times in 50% alcohol first, and then in 70% alcohol. Traces of mercuric chloride were finally removed by treatment with Lugol's iodine solution. The Bouin material was washed in a few changes of 50% alcohol and then in 70% alcohol and the traces of picric acid that may still remain were finally removed by the addition of a few drops of saturated solution of lithium carbonate in 70% alcohol. After washing, the material was preserved in 70% alcohol.

Smear preparations were also made by killing the material on slides previously smeared with a thin coating of Mayer's albumen as per proto-zoological methods (McClung, 1937, pp. 530–31).

The following stains were employed :—Heidenhain's iron-alum hematoxylin, safranin, Mayer's hæmalum, Newton's gentian violet and

safranin and light green. Iron-alum hematoxylin gave the best results. Good results were also obtained by counterstaining the hematoxylin preparations with ½% erythrosin in 95% alcohol.

The procedure adopted for staining in iron-alum hematoxylin is as follows :—A small quantity of the material preserved in 70% alcohol was spread on slides previously smeared with a thin coating of Mayer's albumen and, before the material dried completely, the slide was placed in 85% alcohol. The slight coagulation of the albumen helps to fix the material firmly to the slide. The slides were then brought down the alcohol grades to 30% alcohol and then bleached in 30% alcohol containing 10% hydrogen peroxide. Bleaching in chlorine is not suitable since the material drops away from the slide. The slides were kept in this for 30 minutes and then washed thoroughly in running water for 15 minutes. Bleaching gave a better contrasted stain.

After washing, the material was mordanted in 4% iron-alum solution for one hour, washed in running water for 15 minutes, stained in ¼% hematoxylin for 6–12 hours and differentiated in saturated solution of picric acid. The slides were next washed overnight in running water and passed through the alcohol grades into absolute alcohol, then into clove oil and then into xylol and finally mounted in neutral canada balsam dissolved in xylol.

For counterstaining, the slide from 95% alcohol was placed in a ½% solution of erythrosin in 95% alochol for about 10 seconds and passed rapidly into absolute alcohol and clove oil, cleared in xylol and mounted in neutral canada balsam.

Material stained for 3 hours in Mayer's hæmalum diluted to about one-fourth the strength and washed overnight in running water also gave good preparations. This did not require any differentiation.

Description of the Cell

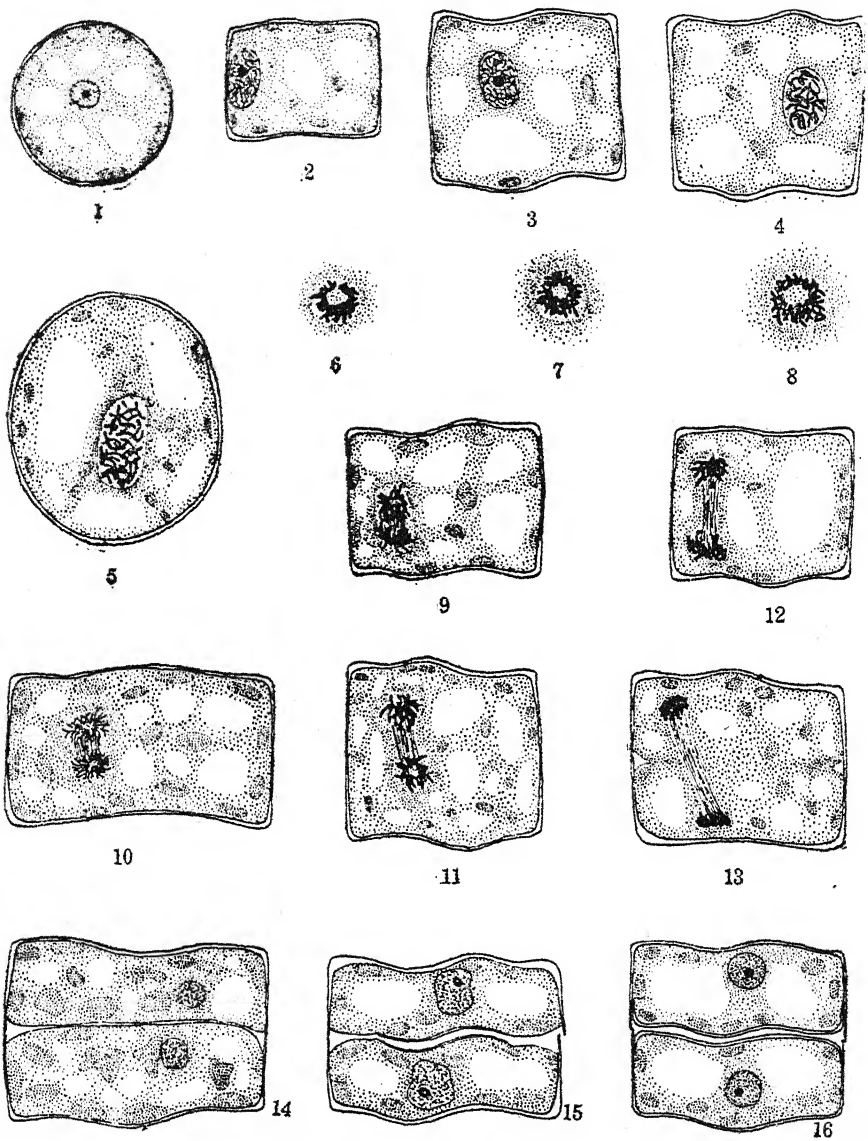
The cells of *Cyclotella Meneghiniana* (Pl. VIII, Fig. 1) are disc-shaped in valve view (Text-fig. 1) and rectangular in girdle view (Text-fig. 2) with an undulation on each of the long sides. In valve view the margin appears striated radially (Pl. I, Fig. 1). The cytoplasm forms a thin lining layer close to the wall and the nucleus is either embedded in this lining layer of cytoplasm or remains suspended by cytoplasmic strands at the centre of the cell. The chromatophores which are lobed or disc-shaped are yelowish-brown in colour (Text-figs. 1 and 2).

Vegetative Multiplication

The diatom multiplies by successive division. Division generally takes place in the early hours of the morning.

Somatic mitosis

The resting nucleus (Text-fig. 1) measures about 4μ in diameter and has generally one nucleolus. It enlarges slightly prior to division. The nucleus during division is generally situated in the parietal layer of cytoplasm. Cholnoky (1933*b*) also found a movement of the nucleus towards one side during division in *Melosira arenaria*.



Text-figs. 1–16. *Cyclotella Meneghiniana* Kütz.—Fig. 1. Resting nucleus. Cell in valve view. (×1120). Figs. 2 and 3. Early prophase. Cells in girdle view. Fig. 2 (×1120); Fig. 3 (×1520). Fig. 4. Late prophase. Cell in girdle view; the nucleolus already disappeared (×1520). Fig. 5. Late prophase. Cell in valve view (×1520). Figs. 6–8. Metaphase, polar view. Note arrangement of chromosomes in a ring around the spindle. In Fig. 6, the gap in the chromosome ring is clearly seen. Note spindle seen in the polar view as dots inside the chromosome ring (×1520). Fig. 9. Early anaphase (×1520). Fig. 10. Early anaphase. Note peculiar dark body on the spindle (×1120). Figs. 11–12. Late anaphase

(×1520). Fig. 13. Early telophase. Note beginning of cytokinesis (×1520). Fig. 14. Division of cytoplasm completed (×1120). Figs. 15–16. Formation of new valves. Figs. 15–16. Formation of new valves ; in Fig. 15 beginning of valve formation seen at the central portion ; in Fig. 16, the valve formation is complete (×1120).

In the resting nucleus the reticulum takes a light stain. The chromatin granules could be seen rather sharply stained in the reticulum (Text-fig. 1). In early prophase the chromosome threads are thin and long (Text-figs. 2 and 3). In late prophase they become somewhat contracted and thicker and are seen lying in the nuclear cavity (Text-figs. 4 and 5). The nucleolus which is visible up to this stage disappears completely now (Text-figs. 4 and 5).

In metaphase the chromosomes are seen arranged in a ring around the equator of the spindle (Text-figs. 6, 7 and 8). The side view of metaphase was not observed. Only the polar view was obtained in the preparations. In the polar view, the fibres of the spindle could be seen as a number of dots at the centre of the chromosome ring (Text-figs. 6–8). At this stage no trace of the nuclear membrane is evident, though a clear space is seen in its place. The chromosomes are rod-shaped, V-shaped and V-shaped with unequal arms (Text-figs. 6–8). The chromosome number could not be definitely determined owing to their large number and very small size and their very compact arrangement in the ring. The number appeared to be more than 60.

The anaphase figures are characterised by long trailing chromosomes which lie more or less parallel to the axis of the spindle (Text-figs. 9 and 11). The chromosomes after reaching the poles of the spindle contract and become compacted together (Text-figs. 12 and 13). In properly differentiated preparations, the outline of a few chromosomes could still be seen in the clumps. In one single instance a darkly stained body was observed in anaphase lying between the two groups of daughter chromosomes on the spindle (Text-fig. 10). The nature of this body could not be determined.

In telophase (Text-fig. 14) the chromosomes gradually become reorganised into the reticulum (Text-figs. 15 and 16).

Cytokinesis

Cytokinesis takes place by the furrowing of the cytoplasm. At about anaphase, a small cleavage furrow is seen starting from the two sides in the girdle view (Text-fig. 13). This cleavage furrow advances inwards in a centripetal manner. During telophase, the furrow advances still further inwards and finally cuts through the spindle fibres and cytokinesis becomes complete. After cytokinesis is completed, the two daughter nuclei are seen very close to each other. Each of the two daughter protoplasts then secretes a new valve on its inner side which fits inside that of the respective mother valve (Text-figs. 15 and 16). The two cells separate soon after.

A small point may be mentioned in this connection. Prior to division, the cells seem to increase in volume by the valves loosening slightly to allow for the increase. Cholnoky (1933*b*) also has recorded

a similar increase in the volume of the cell prior to division in *Melosira arenaria.*

In agar cultures, owing to the daughter cells not separating from each other soon after cell-division, the diatom assumes a filamentous condition, which, however, is soon lost when it is transferred to a liquid medium.

AUXOSPORE-FORMATION

There appear to be so far only very few records of auxospore-formation in the genus *Cyclotella.* The first record of auxospore-formation in this genus appears to be by Thwaites in *C. Kützingiana* (Thwaites, 1848, p. 166, 169, pl. XI, fig. D. 1–5), and later on by W. Smith (1856, vol. II, p. x, pl. B, fig. 47, I–IV) in the same diatom. Hofmeister observed auxospore-formation in *C. operculata* Kütz. in 1857. Later Miquel (1891–92) recorded auxospore-formation in *C. compta* and Bachmann (1904) in *C. bodanica* var. *lemanica.* Hustedt (1930, p. 100) states that auxospores are formed in *C. Meneghiniana* but does not give any details. In all these cases the observations regarding auxospore-formation were very meagre and superficial and no cytological details were attempted.

In the case of the present diatom, plenty of auxospore-formation was observed in the laboratory cultures. An intensive study of the auxospore-formation was made and a detailed account of this process is given here below.

The diatom when inoculated into the culture medium (liquid or agar medium) shows a period of rapid multiplication by vegetative division. This rapid multiplication lasts only for a short time and soon the rate of multiplication slackens and the diatom finally stops dividing altogether. If, at this time, the culture medium is changed, the diatom divides rapidly again for some time and then again stops multiplying. If it is again changed into a fresh culture medium, it begins to divide again rapidly. In this way, repeated transference to fresh culture media helps to keep up the multiplication of the diatom. But, soon there comes a stage when the diatom does not any further respond to changes of the culture media, and for days together no further increase of cells could be noticed, and the diatom begins to die gradually. But, if the diatom cells, when beginning to deteriorate, are transferred to sterilized pond water or to sterilized pond water slightly diluted to about 10–20% with sterilized distilled water, they continue to live healthily and do not degenerate. They do not, however, show any division. At this stage, after some time, they begin to show auxospore-formation. This auxospore-formation was more common in the diluted pond water than in the undiluted pond water. But, only a slight dilution (*i.e.*, 10–20%) is favourable for auxospore-formation. For, if the pond water is diluted to more than 20% with distilled water (say 30%, 40% and 50%), the diatom degenerates and dies out rapidly.

It may be mentioned in this connection that some of the previous workers also found that, when the cells have reached a minimum size,

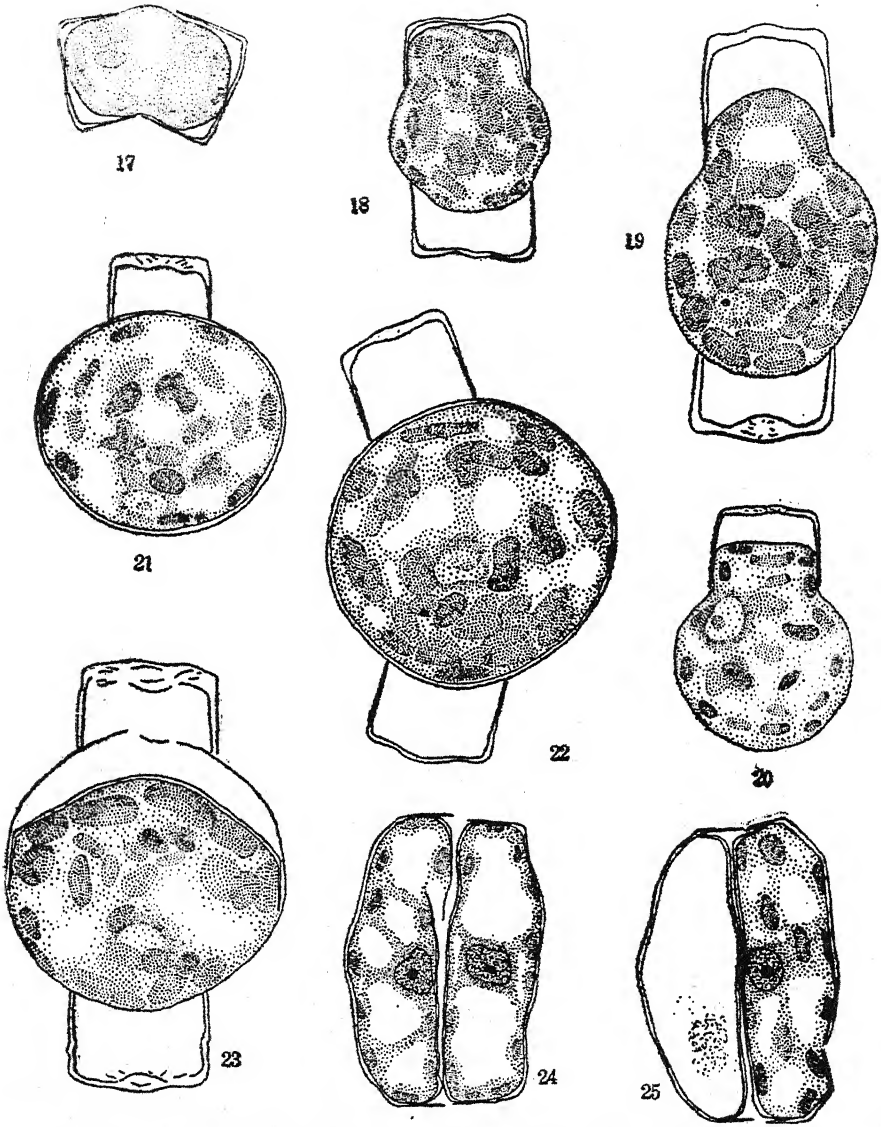
a slight dilution of the culture medium induced auxospore-formation. Schreiber (1931) observed in the case of *Melosira nummuloides* that the cells which have reached a minimum size, when transferred to sea water of a slightly lower concentration, formed auxospores, but, when transferred to sea water of a higher concentration, exhibited abnormalities. He also found that such of the cells as had not yet attained the minimum size, did not form auxospores when transferred to a medium of lower concentration. Geitler (1932, p. 194) found in the case of *Navicula seminulum* that transference to a medium of higher concentration definitely hindered auxospore-formation. He also found in his investigations on several pennate diatoms that the cells formed auxospores only after attaining a certain minimum size. Gross (1937–38 pp. 25, 26 and 45) found that if the cells of *Ditylum Brightwellii* after reaching a certain size are transferred from sea water ' Erdschreiber ' solution to pure sea water (which is a medium of slightly lower concentration), they formed auxospores. Earlier Cholnoky (1928, p. 26) had noticed in the case of *Anomœoneis sculpta* E-Cl. (Pennatæ) that a sudden decrease in the concentration of the medium brought about auxospore-formation. He, however, does not mention anything regarding the size of the cells when forming auxospores.

It was mentioned above that the diatoms form auxospores only after reaching a miniumm size. The same phenomenon was observed by the previous workers also. Pfitzer (1871, pp. 155–56) states that in the case of several diatoms a minimum size should be reached for auxospore-formation. Bachmann (1904) found the same thing in the case of *Cyclotella bodanica* var. *lemanica.* As mentioned already Schreiber (1931), Geitler (1932, 1935) and Gross (1937–38) also found the same thing in the forms investigated by them.

In the present diatom the minimum size for auxospore-formation is generally 10–15 μ in diameter, *i.e.*, about one-third the maximum size of the diatom. But, it was rather peculiar that all the individuals which have become smaller than this size (*i.e.*, below 10 μ in diameter) did not form auxospores but, degenerated and died. In this connection it may be mentioned that Geitler (1932, 1935) also found in the case of several Pennate diatoms that individuals which become very small in size generally died and did not take any further part in the life-cycle.

Description of the process in the living material

In *Cyclotella Meneghiniana* auxospore-formation takes place in the early hours of the morning. The two valves move apart with a slight jerk, which could be seen very clearly when the diatom is watched under the microscope (Text-fig. 17). This sudden jerk is probably brought about by a rapid increase in the turgor pressure inside the cell. After the valves have thus moved apart, the protoplast gradually emerges out from the valves (Text-figs. 18–20 ; Pl. VIII, Fig. 2). When emerging out, it is seen surrounded by the perizonium, the inner pectic layer of the diatom (Text-figs. 21, 22 ; Pl. VIII, Fig. 2). During this process the protoplast escapes out of one of the valves first and then from the other valve (Text-figs. 18–20). As it comes out,



Text-figs. 17–25. *Cyclotella Meneghiniana* Kütz.—Figs. 17–23. Auxospore-formation as seen in the living specimens. Fig. 17. Valves just pushed apart by the enlarging protoplast (×850). Figs. 18–20. Protoplast emerging out of the valves and enlarging during the process. Note the contents emerging out of one of the valves first. In Fig. 20, one valve has dropped off. Figs. 18 and 19 (×1120); Fig. 20 (×850). Figs. 21–22. The enlarged protoplast completely outside the valves and surrounded by the perizonium. (The second valve has already dropped off in Fig. 21.) Fig. 21 (×850); Fig. 22 (×1120). Fig. 23. Formation of the first valve (epitheca) inside the perizonium (×1120). Figs. 24–25. Two daughter cells which were formed after the first division of the new cell. Note the degeneration of one of the two daughter cells in Fig. 25 (×1120).

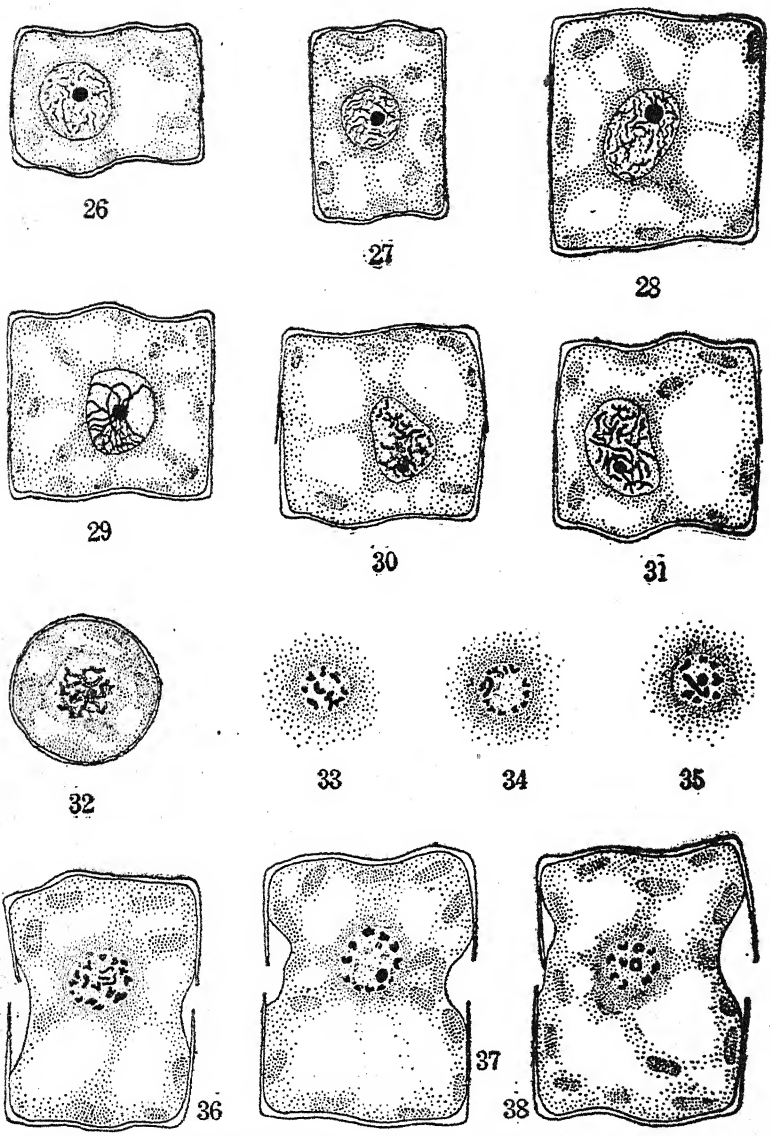
it enlarges gradually and finally becomes very much swollen with the two valves attached to it at the opposite sides (Text-figs. 19–22 ; Pl. VIII, Figs. 2, 3). Occasionally one of the valves may be seen a little displaced to one side (Text-fig. 22 ; Pl. VIII, Fig. 3) or even dropping off (Text-figs. 20, 21). The chromotophores are distributed near the periphery of the swollen protoplast.

The swollen protoplast then contracts from one side of the perizonium (viewed from the girdle view of the mother valve) and secretes a curved siliceous valve, the epitheca (Text-fig. 23 ; Pl. VIII, Fig. 4). It then contracts from the opposite side also and secretes a second valve, the hypotheca, which fits into the epitheca. The characteristic markings of the diatom soon become evident on the two valves. The cell when completed is situated in the middle of the perizonium, its two valves being generally more or less parallel to those of the old auxospore-mother-cell valves. In girdle view the cell is somewhat plano-convex, the epitheca being more convex than the hypotheca, which shows the characteristic undulation of the diatom (Text-fig. 57). The perizonium finally gets ruptured and the new cell is liberated (Text-fig. 57). The whole process of auxospore-formation takes about five hours to complete.

The new cell has a diameter varying from 38–45 μ. This is about three times the diameter of the auxospore-mother-cell. This ratio of the diameter of the original mother-cell to that of the new cell agrees with that recorded by Müller (1906) for *Melosira italica* (Fritsch, 1935, p. 620), by Schütt (1889) for *Chætoceros* and Schulz (1930, p. 28) for *Thallassiosira baltica* (Grun.) Ostenfeld.

Nuclear Changes accompanying Auxospore-formation

The resting nucleus of the cell which is to give rise to an auxospore has a well defined nuclear membrane, a lightly staining reticulum and a single nucleolus. This nucleus is in no way different from that of the ordinary vegetative cell in appearance. It divides twice and forms four nuclei. The first of these two divisions is a reduction division. During the prophase of this reduction division the nucleus increases to almost double its normal size (Text-fig. 26). Thin chromosomal threads become discernible in the nucleus (Text-figs. 26–28). At the next stage (synizesis) the threads are seen more contracted and thicker and lying on one side of the nuclear cavity (Text-fig. 29 ; Pl. VIII, Fig. 5). The free ends of several chromosomal threads are clearly visible and their paired nature can be made out on careful examination. In the next stage (pachytene) the paired chromosomes are seen distributed more uniformly in the nucleus and are thicker and shorter than in the previous stage (Text-fig. 30, 31 ; Pl. VIII, Fig. 7). The bivalents then become still shorter and exhibit their paired nature very clearly (Text-fig. 32 ; Pl. VIII, Fig. 6). The next stage observed in the preparations was diakinesis (Text-figs. 33–35, 36–38 ; Pl. VIII, Figs. 8, 9 and 10). During this stage the bivalents are seen distributed near the periphery of the nucleus. The number of bivalents appears to be about 32–34 (n). The nuclear membrane and the nucleolus disappear soon after diakinesis (Text-figs. 33–38 and 39).



Text-figs. 26–38. *Cyclotella Meneghiniana* Kütz.—Figs. 26–28. Early prophase of reduction division (×1400). Fig. 29. Synizesis (×1400). Figs. 30–32. Pachytene (×1400). Figs. 30 and 31 in girdle view; Fig. 32, in valve view. Note the much shortened and thickened bivalents. Figs. 33–35. Diakinesis in three different foci: Fig. 33, in upper focus: Fig. 34, in median focus and Fig. 35, in lower focus. Nucleolus seen in Fig. 35. In Figs. 34 and 35, one of bivalents is still uncontracted. (×1400). Figs. 36–38. Diakinesis in another cell in three different foci: Fig. 36 in upper focus; Fig. 37, in median focus; and Fig. 38, in lower focus. Nucleolus seen in Fig. 37 (×1400).

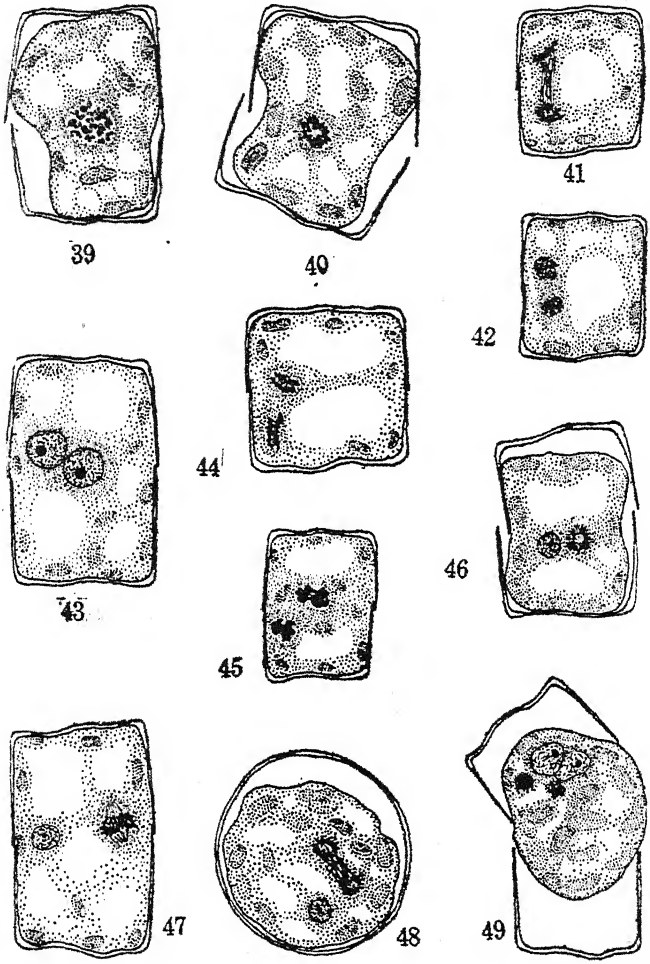
During metaphase the bivalents are seen very compactly arranged in a ring around the spindle (Text-fig. 40 ; Pl. VIII, Fig. 11). After anaphase (Text-fig. 41 ; Pl. IX, Fig. 12) and telophase (Text-fig. 42) two daughter nuclei are organised (Text-fig. 43). The two nuclei next enter into the homeotypic division either simultaneously (Text-figs. 44–45 ; Pl. IX, Figs. 13 and 14) or successively (Text-figs. 46–48 : Pl. IX, Figs. 15 and 16) and form four nuclei. Persidsky (1935) found that in *Melosira varians* also one of the two nuclei sometimes divided later than the other in the second division.

By the time the homeotypic division is completed, the protoplast with the four nuclei emerges outside the valves and becomes very slightly increased in size. Two of the four nuclei degenerate and the remaining two are normal and healthy. Text-figs. 49 and 50, and Pl. IX, Fig. 17 show two healthy nuclei and two degenerating nuclei. Each of the two degenerating nuclei appears as a darkly stained mass. It may be mentioned in this connection that no case was observed in which all the four nuclei were normal. In every case observed, two of the four nuclei had already degenerated. The degeneration of the two nuclei presumably sets in very early after the second division, since in all the cases observed two nuclei had already degenerated. In one case, during second division, one of the groups of daughter chromosomes in each of the division figures appeared to be smaller and more compact than the other (Text-fig. 45). Whether this has any significance in relation to the early degeneration of the two nuclei could not be determined with certainity. It is just possible that the smaller and more compact group of chromosomes in each of the two division figures represents the degenerating daughter nucleus. The healthy nuclei then approach each other and lie in close contact with each other (Text-fig. 50 ; Pl. IX, Fig. 17) and then ultimately fuse (Text-fig. 51 ; Pl. IX, Fig. 18). These two nuclei according to Geitler (1932, p. 10) form the ' gametic nuclei '. Each of these two gametic nuclei always shows a single nucleolus (Text-figs. 49 and 50 ; Pl. IX, Fig. 17). The fusion nucleus[2] shows two nucleoli (Text-figs. 52–55 ; Pl. IX, Figs. 19 and 21) and this condition is seen even after the formation of the valves (Text-figs. 56 and 57). This fusion nucleus forms the nucleus of the new diatom cell formed by the auxospore.

It may be mentioned here that the two gametic nuclei at the time of fusion are in early prophase (Text-figs. 50, 51). The fusion nucleus immediately after fusion is likewise in prophase condition (Text-figs. 52–55). This is in agreement with the observations of Cholnoky (1928, 1929, 1933*a*) on several pennate diatoms, where he found that the gametic nuclei were in prophase condition prior to fusion and so also was the fusion nucleus immediately after fusion. In another diatom, *Melosira arenaria*, one of the Centrales, he (1933*b*) found a large nucleus in prophase during auxospore-formation and presumed that it must have resulted from the fusion of two gametic nuclei. It may be mentioned here that even in the case of some higher plants

[2] ' Syncaryon ' (Geitler, 1935, p. 159). ' Copulatory nucleus ' (Persidsky, 1935, p. 129).

the nucleus of one or both the fusing gametes has been known to attain a prophasic condition during fusion (Sharp, 1934, pp. 236–37).



Text-figs. 39–49. *Cyclotella Meneghiniana* Kütz.—Fig. 39. Late diakinesis. All the bivalents shortened. Note nucleolus and nuclear membrane disappeared (×1400). Fig. 40. Metaphase of I division. Note chromosomes arranged in a ring (×1400). Fig. 41. Anaphase (×1400). Fig. 42. Telophase (×1400). Fig. 43. Two-nucleate stage after I division (×1400). Fig. 44. II Division early anaphase. Note both nuclei in division (×1400). Fig. 45. II division anaphase. Both nuclei in division (×1400). Fig. 46. II division. One nucleus in prophase and the other in metaphase (×1400). Fig. 47. II division. One nucleus in early anaphase and the other in prophase (×1400). Fig. 48. II division (valve view). One nucleus in resting condition and the other in late anaphase (×1400). Fig. 49. Four-nucleate stage with two normal and two degenerating nuclei (dark bodies). Note the prophase condition of the two functional nuclei (×1400).

The auxospore in the meantime enlarges to its full extent with the mother valves clinging to the perizonium (Text-figs. 53–55 ; Pl. IX, Fig. 22). The increase in size of the auxospore takes place only after the fusion of the gametic nuclei, the fusion probably acting as a stimulus. Persidsky (1935, p. 129) also noticed that the same was the case in *Melosira varians.*

The two degenerating nuclei gradually become paler and ultimately disappear completely. In some cases they persist for some time even after the formation of the new valves (Text-figs. 56, 57).

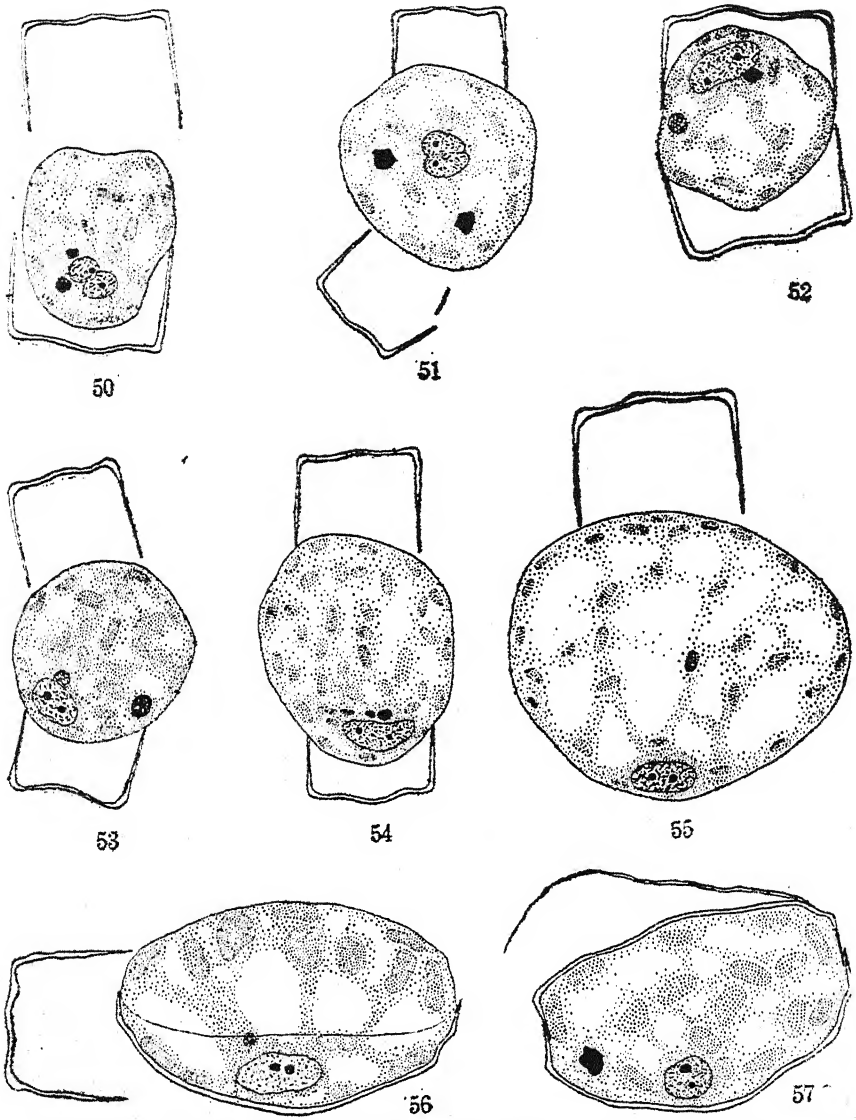
Development of the New Cell resulting from Auxospore-formation

The cell resulting from auxospore-formation is, as already mentioned, somewhat plano-convex in girdle view, the side of the epitheca being slightly convex while that of the hypotheca more or less flat though somewhat wavy (Text-fig. 57). During the division of this cell two new valves with more or less flat sides are inserted inside the old valves with the result that one of the daughter cells (the one which inherits the old epitheca) is plano-convex (Text-figs. 24, 25) while the other (the cell which gets the old hypotheca) is flat on both sides like a normal individual (Text-figs. 24, 25). Two instances of such a division were observed in the material. In one of these, the daughter cell which had inherited the old epitheca was found degenerating, while the other cell was quite healthy (Text-fig. 25). In the other instance both the daughters were quite healthy (Text-fig. 24). Whether the plano-convex daughter cell which is healthy in this case will later on degenerate, it is not possible to state.

The failure of one of the daughter cells of an auxospore to develop normally has been recorded by Geitler (1932, 1935) in the following two members of the Pennales. In *Cocconeis* sp. he found that the first division of the auxospore produces two unequal daughter cells, one of which develops normally, while the other is always irregular in a definite manner and possesses no raphe. The latter is not capable of further development. In *Cymbella sumatrensis*, vegetative nuclear division in the auxospore takes place without the division of the cell contents and one of the daughter nuclei disappears while the other becomes the nucleus of the auxospore which develops normally. With regard to this Geitler (1935, p. 156) states : " An accurate interpretation of this process is not yet possible ; it may be supposed, however, that a preliminary rudimentary cell-division does take place which is represented by the surviving nuclear division ". Such instances, where one of the daughter cells fails to develop do not appear to have been recorded so far among the centric diatoms.

Discussion

Thwaites (1848, p. 166) who is probably the first to study the process of auxospore-formation in the Centrales considers that it is probably a sexual process and states that " there is a great probability of a process taking place in the one cell of the *Melosireæ* precisely



Text-figs. 50–57. *Cyclotella Meneghiniana* Kütz.—Fig. 50. Four-nucleate condition. Same as Fig. 49 (×1240). Fig. 51. Fusion of the two functional gametic nuclei. The dark bodies represent the degenerating nuclei. Note prophasic condition of the fusing nuclei (×1240). Fig. 52. Fusion of the gametic nuclei completed ; fusion nucleus with two nucleoli. Note the prophasic condition of the fusion nucleus (×1240). Figs. 53–55. Protoplast in different stages of enlargement. Note fusion nucleus in all with two nucleoli. In Fig. 55 only one degenerating nucleus seen (×1240). Fig. 56. Formation of the epitheca. Note arched nature of the valve. The mother valve displaced from the usual position. Note also the nucleus with two nucleoli and one degenerating nucleus. Fig. 57. New cells with both valves formed and remnant of the perizonium sticking to it (×1240).

similar in physiological character to the conjugation or mixture of endochromes in other species". He comes to the conclusion mainly on a conjectural basis without any evidence in support of his conclusion (Pfitzer, 1871, pp. 130–31). He states that auxospore-formation in *Cyclotella Kützingiana* (Thwaites, 1848, p. 169) and in the *Biddulphieæ* (*ibid.*, p. 166 foot-note) is quite similar to that of the *Melosireæ*. Other authors following Thwaites, such as Braun (1851), Smith (1856), de Bary (1855–57) and Lüders (1862), supported his interpretation of the process (Pfitzer, 1871, p. 131).

But later authors thought that the process was not sexual but purely asexual or vegetative. Pfitzer (1871, p. 131) who investigated auxospore-formation in *Melosira varians* considered it a purely vegetative process since the auxospore was formed from a single individual.

Müller (1889) who studied auxospore-formation in *Terpsinoë musica* states that it is not a sexual process but only a simple case of rejuvenation of the cell. According to him, auxospore-formation in this diatom takes place in the same manner as the one described by Pfitzer (1871) for *Melosira varians*.

Karsten (1897*b*, pp. 216–17) found that the nucleus of the young auxospores of *Melosira nummuloides* and *M. moniliformis* (*M. Borreri*) contained two nucleoli whereas the nucleus of the vegetative cells had only one nucleolus. He recorded a similar phenomenon in *Sceletonema costatum* (Karsten, 1897*b*, p. 218) also. He supposed that a rudimentary division takes place during auxospore-formation.

Bachmann (1904) who studied auxospore-formation in *Cyclotella bodanica* var. *lemanica* agreed with Karsten in thinking that a supression of cell division takes place during auxospore-formation. He states that, due to external and internal conditions, a sudden increase of turgor pressure occurs leading to auxospore-formation, as a result of which the nuclear division which begins is interrupted, and the cell division is suppressed.

Yendo and Akatsuka (1910) described the auxospore-formation in *Arachnoidiscus Ehrenbergii* as asexual.

Hustedt (1923, 1930) states that the formation of auxospores in *Melosira Jürgensi* Ag. and *M. arenaria* Moore is an asexual process (Hustedt, 1930, p. 115) and states that in *M. Jürgensi* during auxospore-formation there is a suppression of nuclear division as suggested by Karsten.

While all the investigations up to this period have taken it for granted that auxospore-formation in the Centrales is only a vegetative or asexual process, a few very recent investigators (from 1929 onwards) on the Centrales have brought out evidence suggesting that the Centrales are not very different from the Pennales as regards their mode of auxospore-formation.

Persidsky (1929) investigated auxospore-formation in two species of *Chætoceros*, viz., *Ch. boreale* and *Ch. densum*. He found that the nucleus of the auxospore-mother-cell divides twice and forms four nuclei. Of the four nuclei that are formed, two fuse and form the

nucleus of the auxospore, while the remaining two degenerate. He considers the first division as heterotypic, since he claims to have found both "synapsis" and diakinesis stages during this division. Geitler (1931, p. 9) considers the figures given by Persidsky of these stages as quite unconvincing (*cf.* Geitler, 1931, p. 8, figs. 6 *a* and *b*), but considering the similarity of these stages represented by Persidsky to those in the Pennales, he thinks that Persidsky's explanation is probably correct.

Schmidt (1930, pp. 459–61) criticises Persidsky's paper stating that Persidsky was not able to see all the stages continuously. And, he states that none of the earlier workers were able to observe any such nuclear changes during auxospore-formation.

Cholnoky (1933*b*) found in the young auxospores of *Melosira arenaria* one large nucleus and two small degenerating nuclei. The large one becomes the nucleus of the auxospore. He presumes that reduction division takes place before auxospore-formation and that out of the four nuclei that are formed, two fuse and form the large nucleus of the auxospore and the other two degenerate.

Geitler (1934, p. 423) mentions that he found in the auxospores of an undetermined species of *Melosira* one functioning nucleus and one or two degenerating nuclei.

In 1935, Persidsky investigated auxospore-formation in another centric diatom, *viz.*, *Melosira varians.* In this he found that during auxospore-formation the nucleus of the mother-cell undergoes two successive divisions and forms four nuclei. Two of these four nuclei fuse and form the nucleus of the auxospore, while the remaining two degenerate. Of these two nuclear divisions again, he found that the first is definitely heterotypic though he was able to observe only a few stages like synizesis, late anaphase and telophase. The figures given by him in support of the reduction division in this case are more convincing than the ones he gave in support of the reduction division in *Chætoceros* (Persidsky, 1929). Thus the occurrence of reduction division and the fusion of the two nuclei which appeared to be a little doubtful in the case of *Chætoceros* were definitely proved by him to be actual facts in the case of *Melosira varians.* This investigation lends full support to Cholnoky's (1933*b*) and Geitler's (1934) earlier observations mentioned above.

Gross (1937–38) found in the auxospores of *Ditylum Brightwellii* (West) Grun. one large nucleus and two smaller ones and interprets his observations in the same way as Cholnoky (1933 *b*) did in the case of *Melosira arenaria.*

Reith (1940) found during auxospore-formation in *M. arenaria* a large nucleus and a degenerating residual nucleus and states that his observations correspond with those of Cholnoky (1933*b*) on the same diatom.

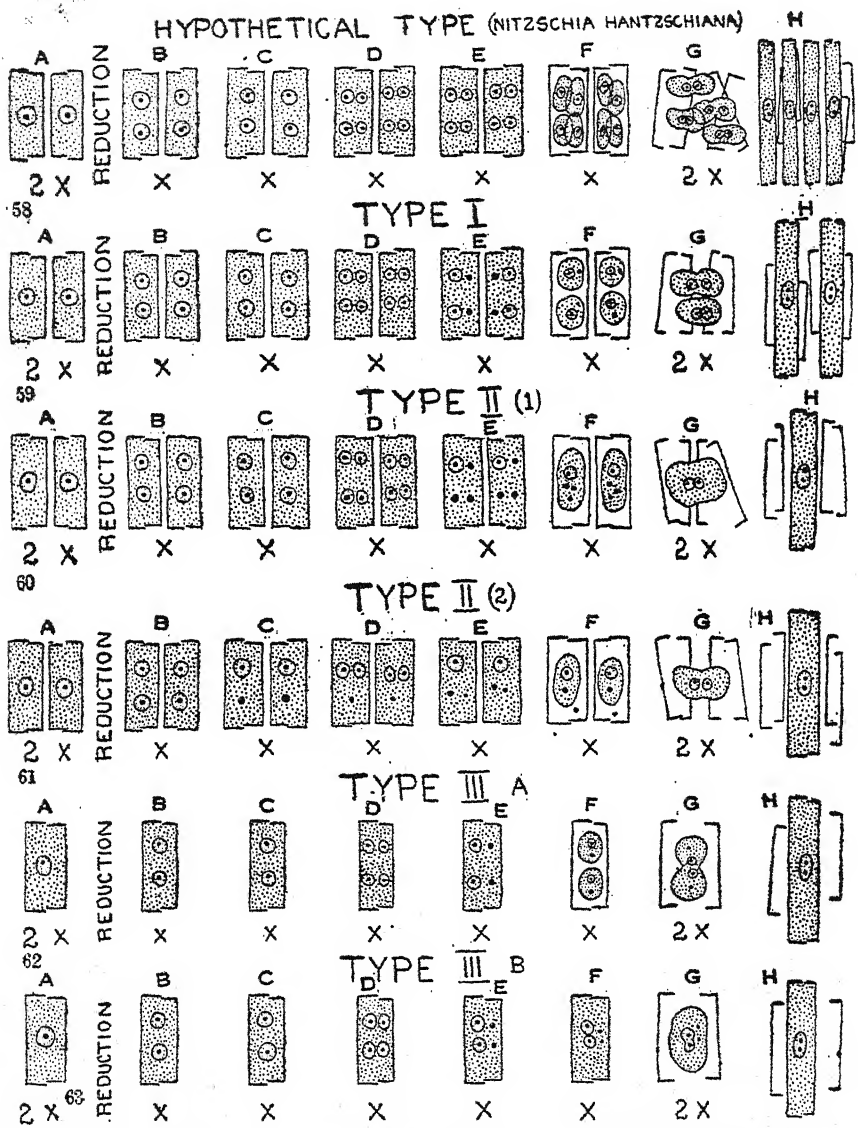
The above-mentioned recent investigations suggest (1) that auxospore-formation in the few members of the Centrales that have been investigated is the result of a sexual process as in the Pennales (though

through the autogamous fusion of two gametic nuclei) and (2) that the vegetative cells are diploid as in the Pennales and undergo reduction division during auxospore-formation.

The observations made by the authors on the present diatom, *Cyclotella Meneghiniana*, are in full agreement with those of Persidsky (1935) on *Melosira varians*. The vegetative phase in the present diatom is definitely diploid. During auxospore-formation the nucleus undergoes two divisions and forms four nuclei. Of these two nuclear divisions the first is definitely meiotic. In this first division almost all the characteristic stages of the meiotic division have been clearly observed. Of the four nuclei that are formed two fuse and form the nucleus of the auxospore, while the remaining two degenerate. Auxospore-formation here is clearly the result of a definite sexual process brought about through the autogamous fusion of two gametic nuclei.

It was mentioned above that Karsten (1897*b*) recorded in the nucleus of the young auxospores of *Melosira nummuloides* and *Melosira moniliformis* two nucleoli and interpreted his observation as indicating a suppression of nuclear division. Geitler (1932, p. 201) criticises Karsten's interpretation and states that the presence of two nucleoli in the young auxospores could on no account be interpreted as indicating a rudimentary division, but, on the other hand, should be considered as due to an autogamous fusion of two nuclei. It is interesting to note that Geitler's interpretation of Karsten's observation is fully borne out by the fact that the nucleus of the young auxospore which is formed through the fusion of the two gametic nuclei in *Cyclotella Meneghiniana* always shows two nucleoli for some time even after the formation of the two valves (Text-figs. 52–57 ; Pl. IX, Figs. 19 and 21).

It was mentioned earlier, that Schmidt (1930, pp. 459–61) criticised Persidky (1929) on the ground that none of the earlier workers who studied auxospore-formation in the Centrales saw any of the nuclear stages recorded by Persidsky, evidently meaning thereby that if these stages really occurred during auxospore-formation, they would not have escaped the notice of the earlier workers. The following are very probably the reasons why the earlier workers failed to observe these nuclear changes. In the case of the Pennales auxospore-formation was known to be a sexual process brought about through the fusion of two gametic protoplasts (*cf.* Pfitzer, 1871 ; Klebahn, 1896 ; Karsten, 1896, 1897 *a*, 1897*b*, 1899, 1900). In the Centrales on the other hand, no such fusion of two protoplasts is noticeable during auxospore-formation and hence this process was considered to be only a vegetative or an asexual process brought about by the mere enlargement of the contents of a vegetative cell. Again, it is only after the protoplast enlarges and begins to emerge out of the valves that the auxospore-formation becomes noticeable. And, by the time the auxospore becomes enlarged and noticeable, all the nuclear changes are already over and the enlarged auxospore shows only one functioning nucleus (the fusion nucleus). As Persidsky (1935, p. 129) states the nuclear changes should be looked for in the small auxospore-mother-cells, which are



Text-figs. 58–63. *Cyclotella Meneghiniana* Kütz.—*Diagrammatic representation of various types of Auxospore-formation in Diatoms.*—Fig. 58. Hypothetical type (*Nitzschia Hantzschiana*). Two pairing cells present; all the four nuclei after reduction division functional; four zygotes formed. Fig. 59. Type I. Two pairing cells present; only two nuclei in each cell functional after reduction division; two zygotes formed. Fig. 60. Type II (1). Two pairing cells present. Only one nucleus in each cell functional after reduction division. Only one zygote formed. Fig. 61. Type II (2). Two pairing cells present. One nucleus extruded out from the cytoplasm after first division of meiosis and of the two nuclei formed in the second

division, one is functional and the other degenerates. Only one zygote formed. Fig. 62. Type III, *a*. No pairing cell present. Auxospore formed from a single cell. Two nuclei functional. The two gametes formed in the same cell fusing and forming one zygote. Fig. 63. Type III, *b*. No pairing cell present. Auxospore formed from a single cell. Division of the cytoplasm suppressed. Of the four nuclei that are formed after reduction division, two degenerate and the remaining two fuse (autogamy); one zygote formed.

hardly distinguishable in external appearance from some of the smallest vegetative cells of the diatom. This the earlier authors evidently failed to do and hence their failure to observe these nuclear changes.

The only person among the earlier workers who came very near to finding out these nuclear stages was Karsten. He (Karsten, 1897*b*) noticed that the nucleus in the young auxospores of *Melosira nummuloides* and *M. moniliformis* had two nucleoli unlike the nucleus of the ordinary vegetative cell which showed only one nucleolus. But, he failed to recognise its real significance, *viz.*, that it is the fusion nucleus in the young auxospore, and so interpreted the phenomenon as a case of suppressed nuclear division.

Four types of auxospore-formation are known in the Diatomaceæ.[3]

In the first type (Text-fig. 59), two individuals come to lie near each other during auxospore-formation (Text-fig. 59*a*). The nucleus in each of the two cells divides twice and forms four nuclei (Text-fig. 59 *d*). The first division is meiotic (Text-fig. 59 *b*). Of the four nuclei that are formed two degenerate (Text-fig. 59 *e*). The protoplast of each cell then divides into two (Text-fig. 59 *f*) and each daughter protoplast receives one functioning and one degenerating nucleus. The daughter protoplasts (gametes) of the two cells fuse and form two zygotes (auxospores), which then increase in size, develop new valves and form two new diatom cells (Text-fig. 59 *g* and *h*). This type of auxospore-formation is seen in *Rhopalodia gibba* (Klebahn, 1896), *Navicula viridula* (Karsten, 1896, 1899), *Cymbella lanceolata* (Geitler, 1927*a*) and *Anomœoneis sculpta* (Cholnoky, 1928), etc.

In the second type (Text-figs. 60 and 61), the protoplast does not divide and so only one gamete is formed in each cell. Two methods of auxospore-formation are seen in this type. In one method (*Surirella splendida*, Karsten, 1900) the nucleus of the cell divides twice and forms four nuclei (Text-fig. 60 *d*). Of these two divisions the first is meiotic. Of the four nuclei that are formed three degenerate while the fourth remains functional and forms the nucleus of the single gamete which is organised in the cell (Text-fig. 60 *e* and *f*). The gametes of the two cells fuse and form a single zygote (auxospore) (Text-fig. 60 *g* and *h*).

In the other method (Text-fig. 61), *e.g.*, *Cocconeis placentula* var. *klinoraphis*, *C. placentula* var. *pseudolineata* (Geitler, 1927 *b*) and

[3] A good account of the different methods of auxospore-formation recorded among diatoms is given by Geitler (1932, 1935). The account given here is based largely on Geitler's account and the nomenclature of the types correspond to those of Geitler.

Navicula seminulum (Geitler, 1932), in each cell one of the two nuclei that is formed in the first division is extruded out with a small quantity of cytoplasm (" Richtungskörper ") (Text-fig. 61 *b* and *f*). The other nucleus divides again and, out of the two nuclei that are formed, one degenerates while the other forms the nucleus of the single gamete which is organised in the cell (Text-fig. 61 *d* and *f*). The two gametes fuse and form a single zygote (auxospore) (Text-fig. 61 *g* and *h*).

In type III (Text-figs. 62 and 63) unlike in types I and II there is no pairing of two separate cells. Two methods of auxospore-formation have been recorded in this type (type III A and type III B). In type III A the contents of a single cell divide into two gametes and the two gametes fuse with each other and form a zygote. In *Synedra ulna* which usually conforms to type I and forms in each cell two gametes which fuse with two other gametes formed in the opposite cell, Geitler (1939) recorded an exceptional instance in which the two gametes formed in a single cell fused with each other and formed a zygote (Text-fig. 62). In *Achnanthes subsessilis* Karsten (1899) found that the protoplast of a single cell divided into two uninucleate portions. In the later stages only one protoplast was seen by him with two nuclei and double the number of chromatophores. He presumes that the two protoplasts have fused. The actual fusion, however, was not observed by him.

In type III B (Text-fig. 63) there is no division of the protoplast but still a single auxospore is formed in each individual. This method is seen in *Amphora Normani* (Geitler, 1928*b*, 1932). In this case the nucleus divides and the resulting two nuclei fuse within the enlarging auxospore. The chromatophore, however, was observed to divide and this division of the chromatophore, according to Geitler, represents a division which has been suppressed. Geitler believes that a second nuclear division also takes place but has been overlooked. This method is a definite case of autogamy. Geitler (1932) includes *Libellus constrictus* (Karsten, 1896) and *Synedra affinis* (Karsten, 1897 *a*) under this method. He includes under this type, the following two centric diatoms also, *viz.*, *Chætoceros boreale* and *Ch. densum* (Persidsky, 1929).

In the fourth type auxospore-formation takes place without sexual fusion (*e.g.*, *Cocconeis placentula* var. *lineata* Geitler, 1927*b*). *Melosira* and other Centrales come under this according to Geitler (1932, p. 213).

Another interesting type may be mentioned in this connection. Pascher (1932, pp. 708–09, fig. 4) found that in *Nitzschia Hantzschiana* the contents of a cell divided into four daughter protoplasts. But no fusion was observed. Nothing is known regarding the nuclear changes connected with the formation of these protoplasts. Since four nuclei generally arise through two meiotic divisions, it is very probable that four nuclei are formed in this case also and each of the four daughter protoplasts receives one of the four haploid nuclei. The four daughter protoplasts very probably represent four gametes. This may, therefore, be considered a case where all the four nuclei remain functional and so four gametes are formed (Text-fig. 58).

A general survey of the different types of auxospore-formation among the diatoms shows that there has been a gradual diminution of sexuality within the group. *Nitzschia Hantzschiana* may be considered to be the most primitive condition. Here all the four haploid nuclei are presumably functional and four gametes are formed in each cell. Type I may be easily derived from a case like *Nitzschia Hantzschiana* through the degeneration of two of the four haploid nuclei that are formed. Here only two gametes are formed in each of the pairing cells.

In type II (Text-figs. 60 and 61) there is a further reduction of the sexual process. Here only one haploid nucleus finally remains functional and consequently only one gamete is organised in each pairing cell. These two gametes fuse and form only one zygote (auxospore).

In type III the sexual process is reduced still further. Here there is no pairing cell and fusion takes place between the products of the same cell. Two methods are seen in this type. In the first method (Text-fig. 62, type III A) two gametes which are formed in a single cell (in the same manner as in type I through the degeneration of two haploid nuclei) fuse with each other and form a single zygote (auxospore). This is a case of *automixis*. In the second method (Text-fig. 63, type III B) the sexual process is still further reduced. Here even the division of the protoplast of the single cell is suppressed, but the nucleus divides twice and forms four nuclei. The first division is presumed to be reductional. Of the four nuclei that are formed, two degenerate and the other two fuse and form a zygote nucleus and a single auxospore is formed. This is a case of *autogamy*.

In type IV sexuality was presumed to be completely absent and that auxospores were formed without sexual fusion of any kind.[4]

Thus we see within the group a gradual decrease in sexuality from a typical normal case as in *Nitzschia Hantzschiana* where all the four haploid nuclei are functional with the result that four gametes are formed to an extremely reduced method of sexual fusion (autogamy) as seen in type III B.

The centric diatoms were until now presumed to come under type IV where sexuality is completely absent and that auxospores in these forms were formed without sexual fusion of any kind. But auxospore formation in the few centric diatoms which have been recently investigated, viz., *Chætoceros boreale*, *Ch. densum*, *Melosira arenaria*, *M. varians*, *Ditylum Brightwellii* and *Cyclotella Meneghiniana* is definitely the result of a sexual process (autogamy) and, therefore, comes under type III B and not under type IV, where auxospores are formed without sexual fusion of any kind as originally believed.

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[4] Geitler (1932, p. 213) includes the centric diatoms *Chætoceros boreale* and *Ch. densum* under type III B, while *Melosira* and other Centrales are included by him under type IV. Recent investigations of Persidsky (1935) and Cholnoky (1933 *b*) have shown that auxospore-formation in *Melosira* also is a sexual process. Therefore Geitler in a later paper (1935) states that excepting in *Melosira*, sexual reproduction among the Centrales is not understood.

It is very interesting that all the few Centrales so far investigated show a sexual reproduction of such an extremely reduced type as autogamy. Further investigation of more members of the Centrales will probably show whether the less reduced types of sexual reproduction seen in the Pennales, *viz.*, types I, II and III A, are also found in the Centrales. In case the other types of reproduction should prove to be absent among the Centrales, then the Centrales should be considered to be a more highly evolved group than the Pennales which still show several less reduced types of sexual reproduction in addition to a few rare cases of autogamy.

In conclusion, the results of the present investigation may be summed up briefly as follows. The vegetative phase in *Cyclotella Meneghiniana* is definitely diploid as in the Pennales, and auxospore-formation is clearly the result of a sexual process as in the Pennales, though the sexual process is of a highly reduced type (autogamy). These observations on *Cyclotella Meneghiniana* are in full agreement with those of Persidsky (1935) made on *Melosira varians* and of Cholnoky (1933*b*) on *M. arenaria*. But Persidsky was not able to observe all the stages of the heterotypic division in *M. varians*. The observations of the other authors are still more meagre. In the present diatom, however, almost all the characteristic stages of the heterotypic division have been observed. The facts brought out in the present and other recent investigations already mentioned would appear to suggest that there is not much fundamental difference between the Pennales and the Centrales. It is very desirable that more members of the Centrales should be investigated in detail as regards auxospore-formation.

SUMMARY

The life-history and cytology of *Cyclotella Meneghiniana* Kütz. a centric diatom, as studied from living and fixed material are described in detail.

Cell-division takes place in the normal manner. The number of chromosomes observed in somatic mitosis appears to be above 60 (2 n).

During auxospore-formation the nucleus divides into four nuclei by two successive divisions, of which the first is a reduction division. Almost all the stages of the reduction division and the fusion of the two gametic nuclei have been followed. The haploid number of the somes appears to be 32–34 (n). Out of the four resulting nuclei two fuse and form the nucleus of the auxospore; the remaining two nuclei degenerate.

It is suggested that the Centrales are not fundamentally different from the Pennales. Here also the auxospore-formation is the result of a sexual process as in the Pennales, only the sexual process in the Centrales is of an extremely reduced type, being completely autogamous. And the vegetative phase is diploid, the haploid phase in the life-history being represented by the four gametic nuclei.

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EXPLANATION OF PLATES

PLATE VIII

Fig. 1. Group of cells from the culture a few days after auxospore-formation; the very large cells are the newly formed cells from the auxospores and the very small cells are the future auxospore-mother-cells. ×80.

Fig. 2. Early stage in auxospore-formation. Contents emerging out of the valves. Note the covering membrane, the perizonium. ×700.

Fig. 3. Auxospore fully enlarged. ×700.

Fig. 4. Auxospore showing formation of the first valve, the epitheca. Note the valve is arched. ×700.

Fig. 5. Synizesis. ×1100.

Fig. 6. Pachytene, seen in valve view. ×1100.

Fig. 7. Pachytene. ×1100.

Figs. 8, 9. Diakinesis in two different foci. ×1100.

Fig. 10. Diakinesis in another cell. ×1100.

Fig. 11. First division metaphase. ×1100.

PLATE IX

Fig. 12. First division anaphase. ×1540.

Fig. 13. Second division early anaphase. ×1540.

Fig. 14. Second division anaphase slightly later than Fig. 15. ×1540.

Fig. 15. Second division metaphase in polar view. The other nucleus is out of focus and has not yet divided. Same as Text-fig. 46. ×1540.

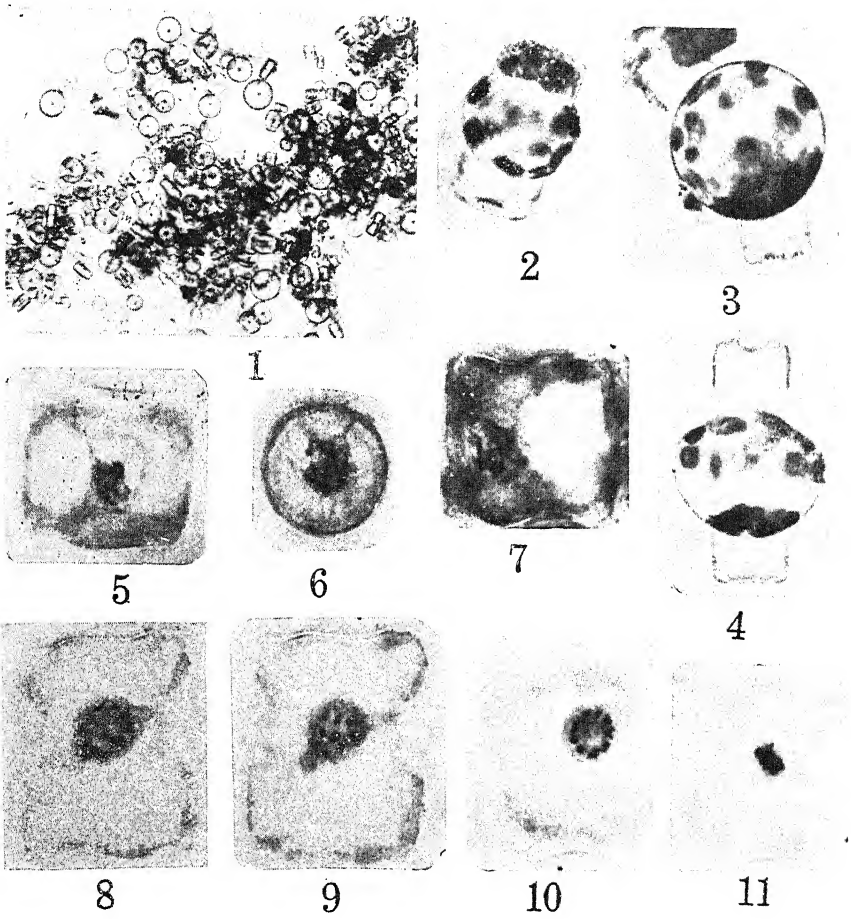
Fig. 16. Second division anaphase (left) in one nucleus; the other nucleus not yet begun to divide. Same as Text-fig. 47. ×1540.

Fig. 17. Four-nucleate stage; two nuclei healthy and two degenerating (seen as dark bodies). Same as Text-fig. 50. ×1260.

Fig. 18. Fusion of the two functional gametic nuclei. Note the two degenerating nuclei seen as dark bodies. Same as Text-fig. 51. ×980.

Figs. 19–21. Protoplast with the fusion nucleus and two degenerating nuclei; note the two nucleoli in the fusion nucleus. Fig. 19, just after fusion. Same as Text-figs. 52, 54 and 53. Fig. 19, ×1540; Figs. 20 and 21, ×1260.

Fig. 22. Protoplast with fusion nucleus (showing two nucleoli); the single degenerating nucleus present is out of focus. Same as Text-fig. 55. × 980.



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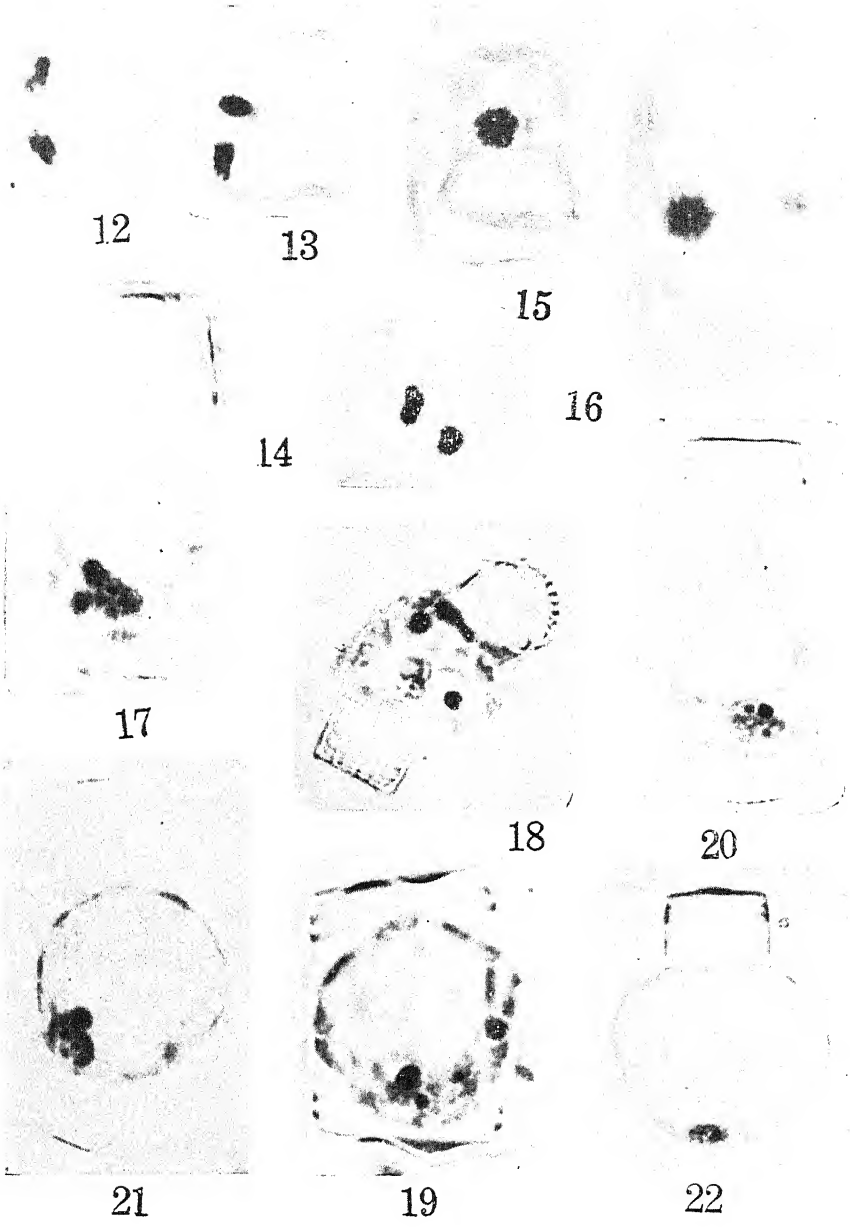
CYCLOTELLA MENEGHINIANA



M. O. P. IYENGAR AND R. SUBRAHMANYAN—

CYCLOTELLA MENEGHINIANA

IMPORTANCE OF ANATOMY IN SYSTEMATICS OF POLYPORACEÆ

By S. R. Bose

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Received for publication on September 15, 1944

In the course of my continuing systematic study of Bengal Polyporaceæ, I have found the following anatomical characters to be of additional help in discrimination of species besides the characters of basidia and spores. Species are grouped under each distinctive character with text-figures (free-hand drawings from hand sections) in some cases.

1. (*a*) Encrusted cystidia

1. *Polyporus zonalis* Berk.
2. *P. violaceo-cinerescens* Petch.
3. *Polystictus elongatus* Berk.
4. *P. abietinus* (Dicks.) Fries.
5. *P. personatus* B. & Br. (Fig. 1).
6. *Lenzites striata* Swartz.
7. *L. adustus* Massee.
8. *L. subferruginea* Berk.

1. (*b*) Simple cystidia (not encrusted)

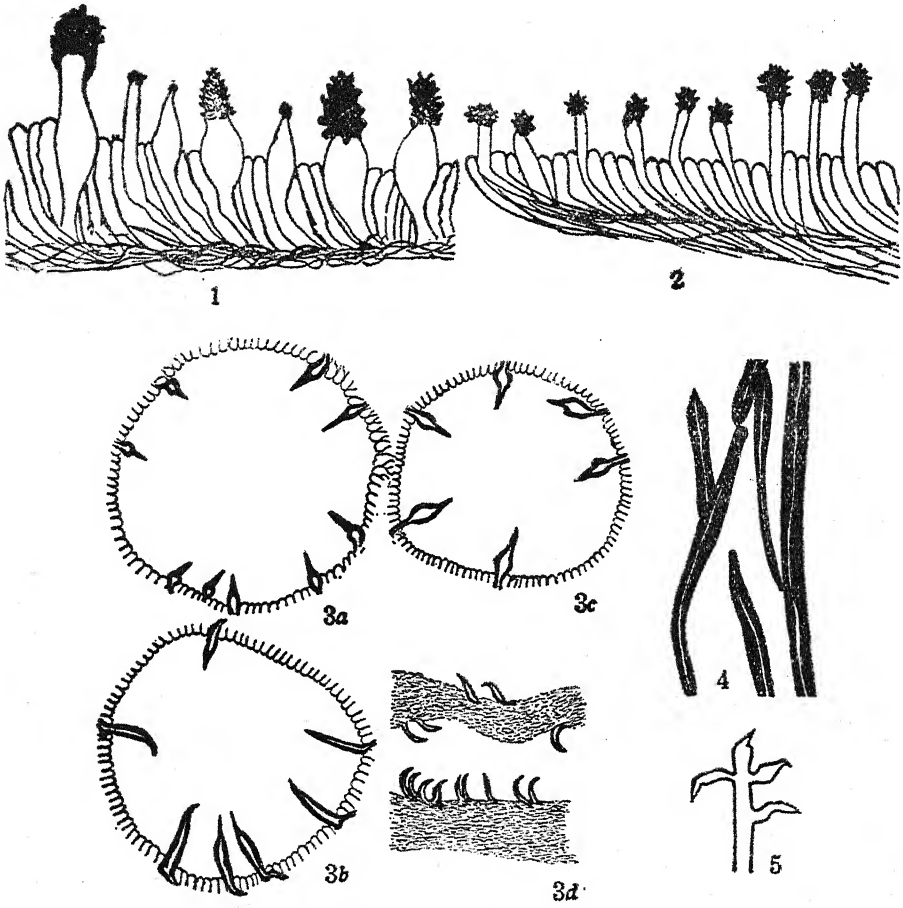
1. *Polyporus agariceus* Berk.
2. *Trametes floccosus* Bres.

1. (*c*) Encrusted hyphæ

1. *Polyporus cervino-gilvus* Jungh.
2. *Trametes versatilis* Berk. (Fig. 2).

2. (*a*) Setæ in the hymenial layer

1. *Polyporus gilvus* Schwein.
2. *P. gilvus* forma *gilvoides* (Schw.) Fr.
3. *P. gilvus* forma *licnoides* (Mont.) Lloyd.
4. *P. cuticularis* (Bull.) Fries.
5. *P. calcuttensis* Bose.
6. *P. hookeri* Lloyd.
7. *P. radiatus* (Schw.) Fr.
8. *Polystictus cichoriaceus* Berk.
9. *P. tabacinus* Mont.
10. *P. xeranticus* Berk.
11. *Fomes conchatus* (Pers.) Fries. (Setæ bulbuos at the base) (Fig. 3 *a*).
12. *F. pachyphlæus* Patouill.
13. *F. lamaensis* (Murr.) Sacc. & Trott.



14. *F. hornodermus* (Mont.) Cooke. = *F. sulcatus* Cooke.
15. *F. senex* Nees & Mont. (Fig. 3 *b*).
16. *F. setulosus* Lloyd.
17. *F. caryophylli* (Rae.) Bres. (Few short setæ in new growths).
18. *F. igniarius* (L.) Fr. (Fig. 3 *c*) (Subulate and ventricose setæ, tubes becoming whitish with deposits of lime with age).
19. *Polyporus circinatus* Fries. (Fig. 3 *d*) (Setæ mostly curved at the apex).

2. (*b*) No setæ in the hymenial layer

1. *Fomes durissimus* Lloyd.
2. *F. fastuosus* Lév.
3. *F. rimosus* Berk.
4. *F. pectinatus* Klotzsch.
5. *F. pinicola* Fr.

6. *F. melanoporus* Mont.
7. *F. fomentarius* (L.) Fr.

2. (*c*) SETÆ EMBEDDED IN THE TRAMA

1. *Polyporus calcuttensis* Bose.
2. *Fomes pachyphlœus* Patouill. (Fig. 4).
3. *F. lamaœnsis* (Murr.) Sacc. & Trott.

2. (*d*) PRESENCE OF CURVED SETÆ ON THE UPPER SURFACE OF THE PILEUS

1. *Polyporus cuticularus* (Bull.) Fries.
2. *P. calcuttensis* Bose (Fig. 5).

3. A HYALINE CELLULAR INTERUPPTED LAYER ON THE UPPER SURFACE

1. *Fomes senex* Nees & Mont.
2. *Polyporus gilvus* forma *licnoides* (Mont.) Lloyd.
3. *P. gilvus* forma *gilvoides* (Schw.) Fr.
4. *P. gilvus* Schwein.
5. *Fomes fastuosus* Lév.
6. *F. pectinatus* Klotz.
7. *F. merrilli* (Murr.) Sacc. et Trott. (Fig. 6).
8. *Polyporus hookeri* Lloyd.
9. *Fomes durissimus* Lloyd.
10. *Favolus scaber* Berk. & Broome.
11. *F. brasiliensis* Fr.

4. RESINOUS PALISADE-LIKE TISSUE ON THE UPPER SURFACE

*1. *Ganoderma lucidus* (Leyss.) Fr. (Fig. 7).
2. *Amauroderma rugosus* Nees.
3. *Fomes subresinus* Murril.
4. *Ganoderma colossus* (Fr.) Bres.

5. NO PALISADE-LIKE TISSUE ON THE UPPER SURFACE, WHICH IS NON-RESINOUS

1. *Ganoderma applanatum* (Pers.) Pat.
2. *Fomes* (*Ganoderma*) *leucophæus* Mont.

By rubbing the upper surface with alcohol it can be seen that *Ganoderma lucidus* and *G. colossus* present a sticky and shining upper surface while *G. applanatus* and *Amauroderma rugosus* and *F. leucophæus* will show a non-sticky and dull (*i.e.*, non-resinous) upper surface.

In *Ganoderma lucidus* when spore-discharge is very brisk, the colour of the hymenial surface is ash gray, then it turns whitish, and when the spore-discharge stops the colour becomes brownish.

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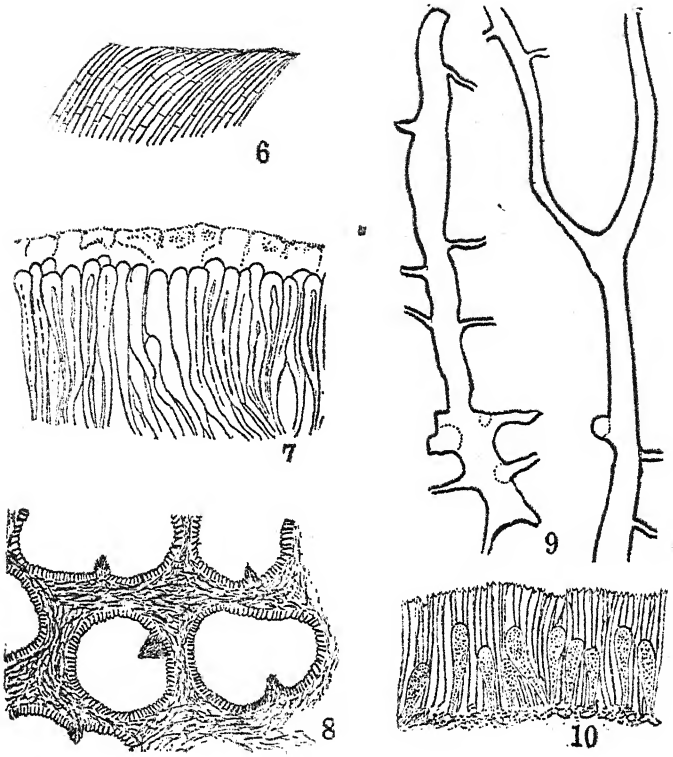
* Bose, S. R., *Mycologia*, Vol. 25. 1933.



6. INDENTED OR LACERATED MARGINS OF THE GILLS AND PORE-MOUTHS

1. *Lenzites striata* Swartz.
2. *Favolus brasiliensis* Fr.
3. *F. scaber* Berk. & Broome.

7. HYPHAL PEGS (CLUSTERS OF CLOSELY AGGLUTINATED HYPHÆ IN THE FORM OF PROJECTIONS) INSIDE THE PORE-TUBES WHICH NEVER BEAR BASIDIA ON THEM

1. *Polyporus thawaitesii* Berk.
2. *P. gramnocephalous* Berk. (pegs of low cone-form) (Fig. 8)
3. *Polystictus hirsutus* Fr. (We find majority of hill specimens of *P. hirsutus* have greater number of hyphal pegs than those of specimens collected from the plains.)
4. *P. sanguineus* (L.) Mey.
5. *P. versicolor* (L.) Fr.
6. *P. vinosus* (Berk.) Cooke.
7. *Favolus brasiliensis* Fr. (pegs of extremely low cone-form)
8. *Trametes serpens* Fr. (Poria)

9. *Hexagonia apiaria* Pers.
10. *H. discopoda* Pat. & Har.
11. *Dædalea unicolor* (Bull.) Fr.
12. *Polystictus zonatus* Fr.
13. *P. xeranticus* Berk.
14. *P. steinheilianus* Berk. & Lév. (pegs of cylindrical form).

N.B.—Some of the hill specimens of *Trametes lactinea* contain a few hyphal pegs in their pore-tubes, while *T. lactinea* collected from the plains does not usually show any hyphal pegs in the pore-tubes.

8. ELONGATED THICK-WALLED CONDUCTING CELLS IN THE CONTEXT AND TRAMA

1. *Polyporus sulphureus* (Bull.) Fr. (Fig. 9).

9. THICK-WALLED AND DEAD FRINGE-HYPHÆ COVERING THE HYMENIUM

1. *Trametes lactinea* Berk. (in some cases).
*2. *Dædalea flavida* Lév. (fringe-hyphæ with bifurcated apices) (Fig. 10).
3. *D. stereoides* Fr. (fringe-hyphæ with tapering apices).
4. *D. quercina* (L.) Pers. (fringe-hyphæ with bifurcated apices).
5. *Hexagonia discopoda* Pat. & Har.

In perennial species of Polyporaceæ growth takes place either (I) from the living hyphal tissue *at the base* of the sporophore, completely covering the entire hymenial surface; thus a stratified sporophore is formed (as in many Fomes, some Polypores, etc.); or (II) from the living hyphal tissue *at the margin* of the sporophore; thus an applante sporophore is produced showing the new zones added year after year.

Specimens of Polyporaceæ can be distinguished from specimens of *Hydnum* by bearing groups of basidia at the bases of pore-tubes in longitudinal section, whereas in *Hydnum* species basidia are not found between the bases of spines, such bases remaining distinct.

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* Bose, S. R., *Ann. Mycol.*, Vol. 36, 1938.

MELANOPSAMMA RANJANII SP. NOV.: A NEW PARASITE OF *SELAGINELLA**

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Received for publication on May 22, 1944

Introduction

The fungi so far recorded on the various species of *Selaginella* have been listed by Gregor (1938) and by Mitra (1943). The specimen to be described here, differs from all the fungi mentioned in the above lists. It was found growing on living *Selaginella chrysocaulos* in a shaded place in the Lloyd Botanical Gardens, Darjeeling, in the month of September 1938. Even after a very careful search, however, not more than two infected plants could be found. But these infected plants showed the black perithecia of the fungus at the tip of almost every branch and spike, many of which had well-developed micro- and megasporangia (Fig. 1). These fructifications were not found on the branches near the base of the plant nor on other parts, such as stem and leaves. The only other external symptom of these plants was a little drooping of the infected branches. They were green and apparently were not killed by the parasite at the stage at which the material was collected. Unfortunately both the infected plants were preserved in formalin-acetic-alcohol, so that no cultural studies or inoculation experiments could be made. The writer had thus to be content with a study based on teasings and microtome sections of the original material.

Observations

Host-Parasite Relation

A longitudinal section through the infected tip (Fig. 1) revealed the very interesting feature that the hyphæ of the parasite were present only in the xylem of the vascular bundle. To ascertain the extent of penetration, transverse sections of the stem at various heights were examined. Sections of the stem at the very base did not show infection. The presence of the fungus was first detected in sections about 1·8 cm. below the lowest branch whose tip bore the perithecia. Above this portion every section up to the very top showed the presence of the parasite.

A careful examination of the transverse section of the infected part of the main stem or the branches (Figs. 3 and 4) shows the presence of hyphæ inside the xylem only, the phloem, pericycle, endodermis and cortex being quite free from the parasite. The hyphæ are found

* Read before the Joint Meeting of the National Academy of Sciences and the Indian Academy of Sciences, held at Hyderabad, 1943.

in quite large numbers in all parts of the xylem, including protoxylem and many of the tracheids are even found to be clogged (Fig. 4). Near the tip of the branches where the xylem breaks up into isolated strands separated by parenchyma, the hyphæ are found inside the xylem as well as the parenchymatic cells, but even here they do not grow into the pericycle or parts of the cortex. At the very tip of the branch, however, all the undifferentiated parenchymatic cells are attacked and this infected part gradually merges into a pseudo-parenchyma formed entirely of fungal hyphæ, on which the perithecia are situated (Fig. 1). The infected tracheids of the stem are continuous with those of the branch trace. The hyphæ also enter the xylem of the leaf trace but do not affect any other part of the leaf (Fig. 1). The growth of the hyphæ inside the xylem evidently interferes with the flow of water and this explains the drooping of the infected branches already referred to. A longitudinal section of the stem (Fig. 5) shows a luxuriant development of the hyphæ within the tracheids. They are fairly thick and frequently show branching, anastomoses and H-pieces.

Description of the Parasite

Except the perithecia no pycnidial or other imperfect conidial stages have been found.

Perithecia.—At the tips of vegetative shoots or sporangiferous spikes one can see minute globose, carbonous perithecia present superficially in groups of two to five, solitary ones being extremely rare. They are hard, smooth and devoid of hairs. These seem to be situated directly on the tips of branches but a longitudinal section through the infected region shows that they are seated on a small pseudoparenchymatous base which does not form a well-marked external stroma. This pseudoparenchymatic base, comes out with the perithecia if they are separated (Fig. 6 A). The venter of the perithecium is spherical (Fig. 2). There is no beak, but the ostiole is situated as a clearly defined pore in a minute round papilla at the top (Fig. 6 A). Inside the perithecium are asci and paraphyses originating from the basal region and the sides. They are absent in the region of the neck where they are replaced by periphyses. The size of the perithecia varies greatly and sometimes younger perithecia are found attached to the same pseudoparenchymatic base as the mature ones. The mature perithecia range from 209 μ to 383 μ in diameter. A perithecium of average size measures about 300 μ.

Asci.—The asci are cylindrical or club-shaped. They have got short, tapering stalks and possess slightly flattened bases for attachment (Fig. 6 B). Each ascus contains eight ascospores generally arranged in a single series (monostichous), but here and there some of the ascospores show a distichous arrangement (Fig. 6 B). The asci are hyaline to somewhat translucent and contain oil globules, which come out when teased. The asci are 80–88 μ long and 12–14 μ broad.

Paraphyses and Periphyses.—Paraphyses are free, persistent, unbranched, non-septate and do not anastomose with each other (Fig. 6 C). They are hyaline to translucent and contain oil globules. The paraphyses are shorter and narrower than the asci, but like them possess a

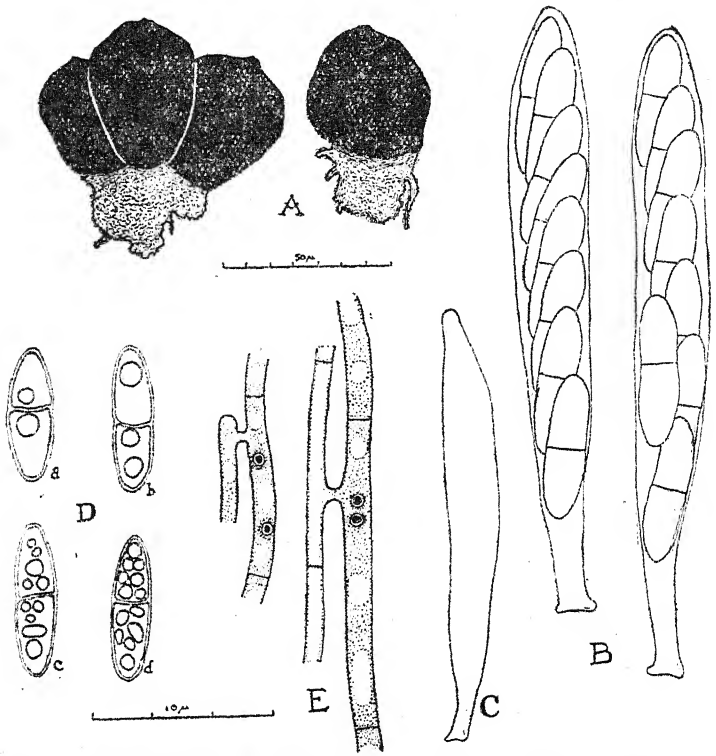


Fig. 6. *Melanopsamma Ranjanii* sp. nov.—Camera lucida drawings of perithecia, asci, etc. Fig. A, Perithecia with basal pseudoparenchymatic mass generally found in groups but in rare cases single. × 64. Fig. B, Asci with monostichous ascospores. × 800. Fig. C, Paraphysis. × 800. Fig. D, Bi-celled ascospores showing stages in maturation and increase of guttulæ in older spores. × 800. Fig. E, Binucleate hyphæ and H-pieces from L.S. of stem in xylem region. × 800.

tapering stalk, a flattened base and a rounded apex. They measure on an average 72 μ × 10·5 μ. The periphyses never come out of the ostiole.

Ascospores.—The ascospores are ovoid to spindle-shaped, sometimes slightly curved, hyaline and bicelled. The equatorial septum divides the spore into two almost equal halves with a very slight constriction in the middle, which may sometimes be absent. Their ends are gradually rounded although in a few cases they are more pointed than usual. At first each cell of the ascospore is uni-guttulate but as it ages the guttulæ increase in number, so that the cells of the mature spore become multiguttulate (Fig. 6 D). This makes the septum difficult to see without proper staining. The ascospores vary from 20 to 28 μ in length and 7 to 8 μ in breadth. The average size of the ascospore is 26 μ × 7 μ.

Mycelium.—The loose hyphæ inside the tracheids are branched and consist of elongated cells which are 30–60 μ long and 2·5–5 μ broad.

They frequently show anastomoses and H-pieces (Fig. 5). These cells contain a number of vacuoles. The cells near the pseudoparenchymatic base are rectangular to iso-diametric.

Cytology.—The mycelium, which shows fusions and H-pieces at various places, shows two nuclei in each cell. These nuclei may lie very close to each other or may be more or less apart (Fig. 6 E). They measure about 2 μ in diameter and consist of a deeply staining central body with a white halo around it. In a few cases very much elongated nuclei were found. These were probably in the course of division. Youngest perithecia are uniformly pseudo-parenchymatous with outer layers of thicker cells. In slightly older ones the centre is occupied by a mass of deeply staining hyphæ, the cells of which are bi-nucleate. The nuclei in this case were much smaller. No definitive nucleus or other stages in the cytology of the ascus were observed. Each cell of the ascospore contains a single nucleus.

Identity of the Fungus.—The superficial, glabrous and unbeaked nature of the carbonous perithecia, which are not situated on a distinct stroma, together with the presence of hyaline, ovoid, bi-cellular spores indicate that this fungus belongs to the genus *Melanopsamma* Niessel (Fam. Sphæriaceæ). The fungus also agrees with the description given for *Melanopsamma* by Saccardo (1887) and by Winter (1887). A large number of species of *Melanopsamma* is known and many of them grow on rather primitive phanerogams, such as the Archichlamydeæ, Gymnosperms, etc. But none has been recorded so far on *Selaginella.* The habitat of the fungus together with the measurements of perithecia, asci, ascospores, etc., show that it is a new species. I have much pleasure in naming it *Melanopsamma Ranjanii* sp. nov. after Dr. Shri Ranjan, Professor of Botany, University of Allahabad. It may be noted here that this is also the first record of *Melanopsamma* on any host in India (*cf.* preliminary note by Mitra, 1943). Neither Butler and Bisby (1932) nor Mundkur (1938) mention anything about this genus. This is rather surprising as species of *Melanopsamma* are well represented in the tropics—a large number having been recorded from the Philippines. Gwynne-Vaughan and Barnes (1922, p. 153) also remark in connection with the Sphaeriales that "there is no doubt that a study of the tropical forms at present very incompletely known, will greatly increase their number."

Discussion

A great deal of difference of opinion exists as to the limits of the Sphæriales. Petrak (1924) has shown that many species till then regarded as simple Sphæriales really belonged to the Pseudosphæriales, which are related to the Dothideales as their perithecia are in reality unilocular stroma. Many modern writers (Miller, 1928; Theissen and Sydow, 1918) have recognised this differentiation and have given certain criteria for distinguishing between a simple perithecium and an unilocular stroma. According to Miller (1928) the attempt by Petrak (1924) and others to separate these two on the basis of thickness of wall is not fruitful. On the other hand he considers the presence of a true perithecial wall to be a fundamental criterion of a true perithecium.

Chesters (1938) agrees with this. Presence of a true perithecial wall is correlated with other characters. The centre of the perithecium is not pseudoparenchymatous, asci do not ripen in series so that ripe and unripe asci are not found together, and true paraphyses and periphyses occur. If we apply the above criteria we see that *Melanopsamma Ranjanii* possesses true perithecia and so belongs to the Sphæriales. Chesters (1938) in his studies on two species of *Melanomma* came to the conclusion that these two species ought to be placed in the Pseudosphæriales, and opines that "It is probable that species of *Bertia* and *Melanopsamma* will be found to have a similar development to that of *Melanomma* and to belong to the Pseudosphæriales." The present study shows that Chesters' (1938) prediction is not true for all the species of *Melanopsamma*.

Diagnosis of Melanopsamma Ranjanii *sp. nov.*

Pĕrithecia superficialia, in summitate ramorum, gregaria, 2–5 simul, spherica, carbonacea, lævia, ostiolum in minuta papilla, 209–383 μ diam.; asci cylindrici vel clavati, octo-spori, hyalini, 80–88 $\times$ 12–14 μ; paraphyses clavatæ; sporidia monosticha, ovoida vel fusoidea, apice rotundo, hyalina, uniseptata, multi-guttulata dum matura, 20–28 $\times$ 7–8 μ; mycelia in xylemo caulis hospitis tantum, sed inficientia alias parenchymaticas cellas summitatis, bi-nucleata, cum frequentes anastomoses et H-partes.

Hab.—In summitate ramorum et spicis *Selaginellæ chrysocaulis*, Darjeeling, India, September 1938.

Perithecia superficial, at tips of branches, gregarious, in groups of 2–5, spherical, carbonous, smooth, ostiole in a minute papilla, 209–383 μ in diameter; asci cylindrical or club-shaped, eight-spored, hyaline, 80–88 $\times$ 12–14 μ; paraphyses clavate; ascospores monostichous, ovoid to spindle-shaped, ends rounded, hyaline, uniseptate, multi-guttulate when mature, 20–28 $\times$ 7–8 μ; mycelia only in xylem of the stem of the host but infects other parenchymatous cells at the tip, bi-nucleate, shows frequent anastomoses and H-pieces.

Hab.—At the tips of branches and spikes of *Selaginella chrysocaulos* in Darjeeling, India, September 1938.

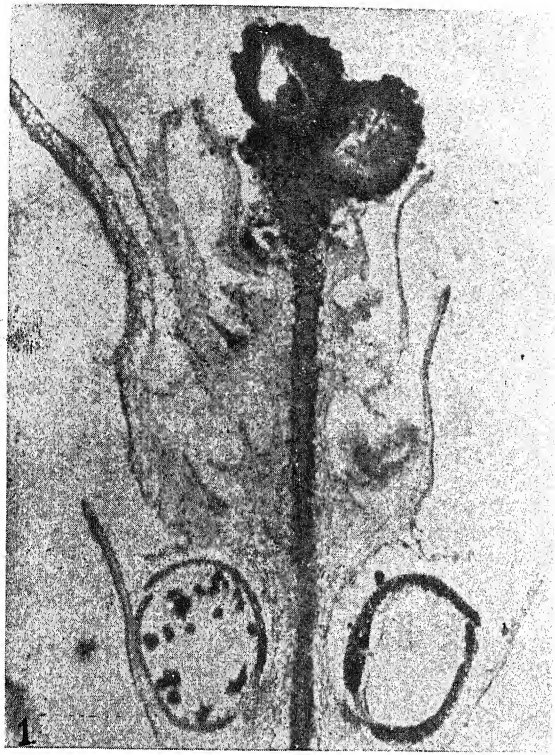
Type specimen deposited in the Herbarium of the Botany Department, Universtiy of Allahabad, India.

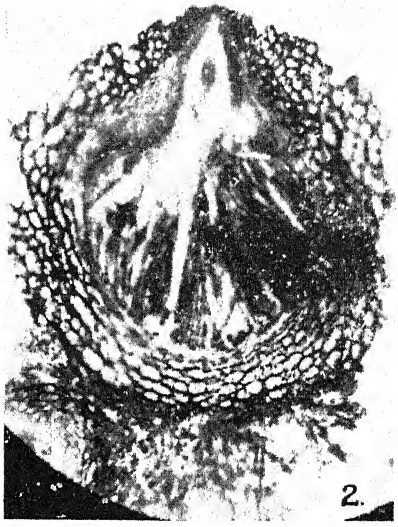
Summary

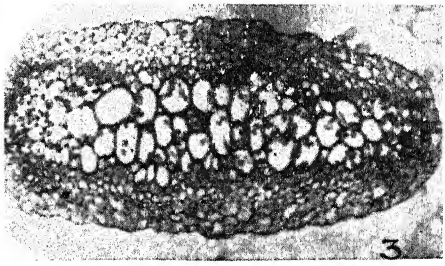
1. *Melanopsamma Ranjanii* sp. nov. is recorded as a new parasite of *Selaginella chrysocaulos*. This is also the first record of *Melanopsamma* on any host in India.

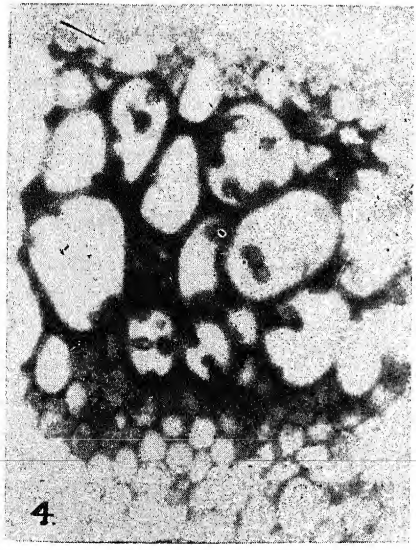
2. The black perithecia occur only at the tips of branches which do not show any other symptom except drooping.

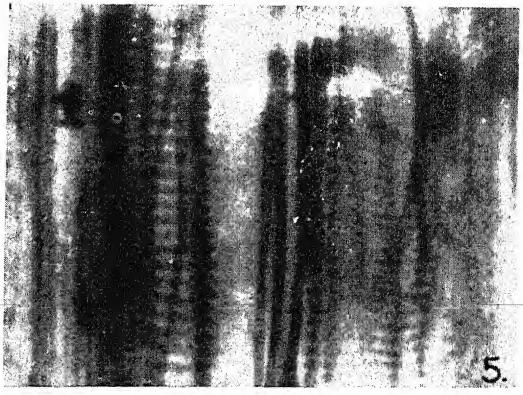
3. In the stem of host, the parasite is present only in the xylem region without affecting other parts. Some tracheids are found to be totally clogged by the parasite. Here many hyphæ showing H-pieces and binucleate cells are found.











A. K. MITRA—*MELANOPSAMMA RANJANII* SP. NOV.

4. At the tip of branches the young parenchymatous cells are attacked and this infected part gradually merges into a pseudo-parenchyma formed entirely of fungal hyphæ.

5. It has been shown that *Melanopsamma Ranjanii* possesses true perithecia and so belongs to the true Sphæriales.

6. A full description of the parasite is given.

In conclusion, the author wishes to express his thankfulness to Professor Shri Ranjan, D.Sc., Head of the Department of Botany and Dean of the Faculty of Science, University of Allahabad, for his valuable suggestions and for kindly going through the slides and manuscript. The author is also indebted to Father Jerome of St. Joseph's Seminary, Allahabad, for help in translating the diagnosis into Latin.

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EXPLANATION OF PLATE

Melanopsamma Ranjanii sp. nov. on *Selaginella chrysocaulos*

Fig. 1. L.S. through tip of an infected spike with mature sporangia of the host, showing terminal perithecia, affected conducting strand of stem and leaves.

Fig. 2. L.S. through a perithecium showing asci, paraphyses, wall and spherical venter.

Fig. 3. T.S. through the vascular bundle of the stem in the infected region showing hyphæ in xylem.

Fig. 4. Hyphæ in xylem. Note their absence in phloem and pericycle.

DEVELOPMENT OF THE EMBRYO-SAC IN THE CONVOLVULACEÆ

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There are a number of contradictory statements in the literature on the embryology of the Convolvulaceæ, particularly with reference to the presence or absence of parietal cells. Peters (1908) investigated *Cuscuta europea* and *Convolvulus sepium* and reported that the primary archesporial cell in both of them cuts off a wall cell and the embryo-sac develops according to the normal type. Asplund (1920) also reported the formation of a primary wall cell in *Cuscuta lupuliformis*. Svensson (1925), on the other hand, holds the view that the parietal cells described in *Cuscuta* and *Convolvulus* by Peters and Asplund were probably derived from the epidermis and are not true parietal cells. Dahlgren (1927) shares the views of Svensson and states that parietal cells are definitely absent in *Cuscuta lupuliformis* and *C. epithymum*. Macpherson (1921) who studied the embryo-sac of *Cuscuta gronovii* and *Convolvulus sepium* could not observe the early stages of its development. Kenyan (1929) investigated *Ipomea trifida*. He found numerous archesporial cells, formation of the parietal cells and a normal type of embryo-sac. His observations also indicate that the inner cells of the integument are consumed during the growth of the embryo-sac. Mathur (1934) reported the occurrence of a definite primary parietal cell in *Convolvulus arvensis*. Johri (1934) found that in *Cuscuta reflexa* the hypodermal archesporial cell functions directly as the megaspore-mother cell and the wall cells are completely absent. He has further stated that the development of the embryo-sac conforms to the *Scilla*-type. Smith (1934) in several species of *Cuscuta* growing in North Carolina and Tiwary and Rao (1936) in *Evolvulus nummularis* found that the embryo-sac develops according to the normal type. They do not say anything about the parietal tissue. Raghava Rao (1940) in a recent paper describes the development of the embryo-sac in *Ipomea Learii*, *I. staphylina*, *I. hederacea*, *Argyreia speciosa* and *Evolvulus alsinoides*. He reports the formation of a primary wall cell in *Ipomea Learii* and its aosence in *I. staphylina* and *Evolvulus alsinoides*. He says nothing about *Ipomea hederacea* and *Argyreia speciosa* in this connection.

The present investigation deals with the development and structure of the embryo-sac in *Jacquemontia violacea* Choisy, *Ipomea pulchella* Roth., *I. Horsfalliae* Hook. *f.*, *I. obscura* Ker-Gawl., *I. sepiaria* Koenig and *Operculina Turpethum* Manso. Material of these was collected from the environs of Bombay and studied according to the customary methods.

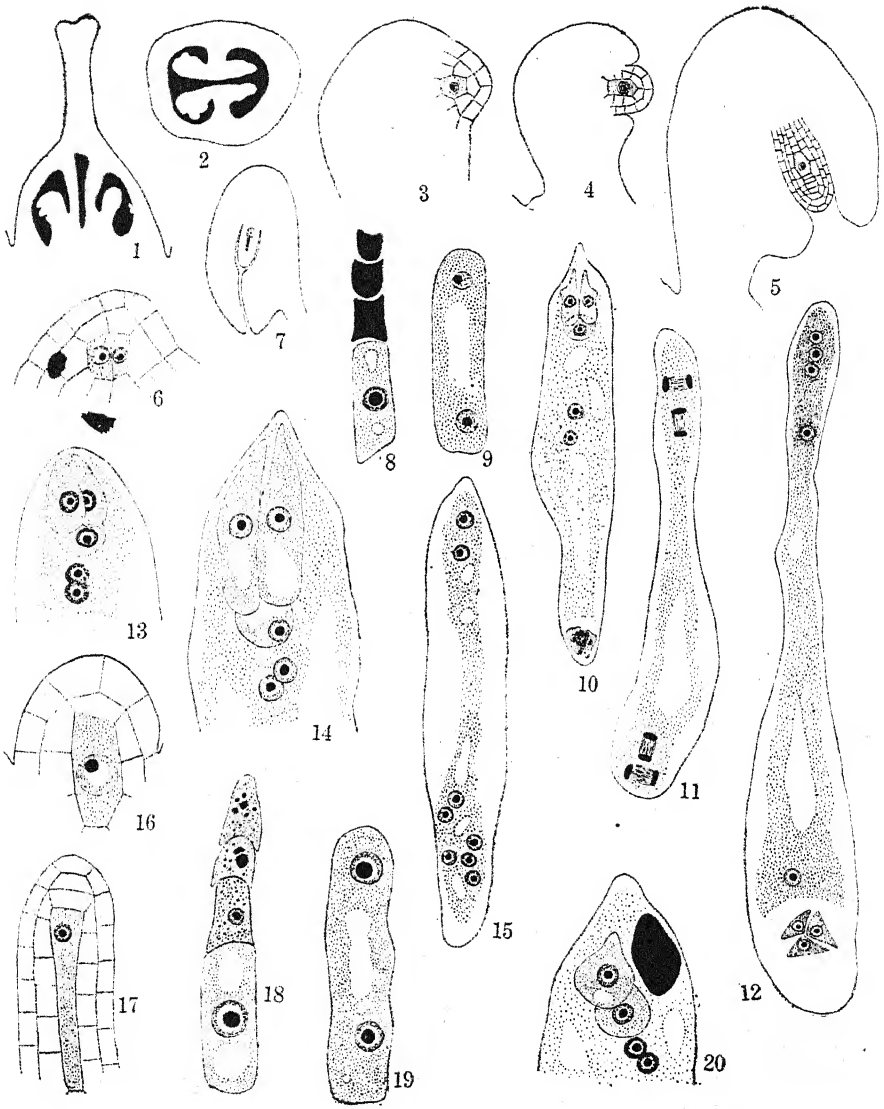
OBSERVATIONS

Ovary and Ovule.—The ovary of the Convulvulaceæ is generally described as bilocular, with two axile ovules in each loculus arising from near the base as shown in Fig. 1. Such a description however is not true for all the stages. Serial transverse sections of young ovaries, as illustrated in Fig. 2 for *Jacquemontia violacea*, reveal that the ovule-bearing carpel margins do not meet in the centre. The ovary therefore is at first unilocular and the placentation marginal and parietal. The two placentas are separated near the base of the ovary by a narrow channel. Near the top of the ovary the margins of the carpels become even more clear and those of the opposite sides are seen to be quite free. The line of fusion between the two carpels is quite clear even in the style. Thus the microscopical examination of the young stages of the ovary shows that the union of the carpels is not so thorough as might appear from the outside. These stages also clearly bring out the bicarpellary nature of the gynoecium.

The ovule initials as usual arise as papilla-like outgrowths from the placenta (Fig. 3). Soon the terminal part of the initial curves so as to make an angle of 90 degrees with the basal part, which forms the funicle of the ovule. It is about this stage that the single integument arises from the base of the nucellus (Fig. 4). The bending of the ovule continues until the adult anatropous form is attained. The funicle is very short. The nucellus is very small as compared with the thick integument. The micropyle is long and extremely narrow as described by Kenyan (1929) in *Ipomea trifida.* This is clear from Figs. 5 and 7, which illustrate the structure of the ovules of *Jacquemontia violacea.*

Development of the Embryo-sac.—The archesporium is hypodermal and differentiates very early, even before the origin of the integument. In *Jacquemontia violacea* sometimes two or three archesporial cells are seen to occur side by side in the same nucellus (Fig. 6). In all other species in every case only one archesporial cell was noted.

The archesporial cell cuts off a parietal cell immediately after its formation (Figs. 3 and 16), which divides further to form a distinct, parietal tissue (Figs. 4, 5 and 17). The first division wall formed in the parietal cell is periclinal (Fig. 17) or anticlinal (Fig. 4). The second division may be either anticlinal or periclinal, and the later divisions occur irregularly. As the result of these divisions in the primary wall cell, the megaspore-mother cell is covered by about 4–7 layers of cells (Fig. 5). Raghava Rao (1940) states that the first division of the primary wall cell in *Ipomea Learii* is anticlinal and a similar behaviour has been observed by Mathur (1934) in *Convolvulus arvensis.* From the occurrence of primary wall cell in every one of the six species investigated during the course of the present work, it appears, in spite of Dahlgren's (1927) strong criticism, that Peters (1908) was correct in his assertion about the occurrence of parietal cells in the Convolvulaceæ investigated by him. Also, therefore, many of the observations on the Convolvulaceæ, where the presence of the parietal cells has been denied, appear to be doubtful and deserve reinvestigation.



Figs. 1–20.—Figs. 1–10. *Jacquemontia violacea.*—Fig. 1. L.S. of a gynœcium with the ovules at the stage shown in Fig. 5. × 60. Fig. 2. T.S. of a young gynœcium slightly above the middle of the ovary. × 60. Fig. 3. A young ovule showing the formation of the primary wall cell. × 440. Figs. 4 and 5. Two ovules showing the megaspore-mother cell and the development of the nucellus. × 260. Fig. 6. An ovule showing two megaspore-mother cells. × 570. Fig. 7. An ovule at the tetrad stage showing the thick integument and the long narrow micropyle. × 260. Fig. 8. A linear tetrad of megaspores with the three micropylar megaspores degenerating. The chalazal one is developing into the embryo-sac. × 950. Fig. 9. A 2-nucleate embryo-sac. × 950. Fig. 10. A mature embryo-sac. × 570. Figs. 11–13. *Ipomea pulchella.*—Figs. 11 and 12. Two stages in the development of the

8-nucleate embryo-sac. × 570. Fig. 13. Micropylar region of a mature embryo-sac, showing the egg-apparatus and the polar nuclei. × 260. Figs. 14–15. *Ipomea Horsfalliæ*.—Fig. 14. Micropylar portion of an embryo-sac showing the egg-apparatus and the polar nuclei. Fig. 15. An abnormal embryo-sac showing 6 nuclei at the chalazal end and 2 at the micropylar end. × 570. Figs. 16–20. *Operculina Turpethum*.—Fig. 16. An ovule showing the megaspore-mother cell and the formation of the primary wall cell. × 950. Fig. 17. An ovule showing a later stage in the development of the megaspore-mother cell. × 570. Fig. 18. A linear tetrad of megaspores. × 950. Fig. 19. A binucleate embryo-sac. × 950. Fig. 20. Micropylar portion of a mature embryo-sac with one of the synergids degenerating. × 440.

The megaspore-mother cell forms a linear tetrad of megaspores by two successive divisions,—*cf.* Fig. 8 for *Jacquemontia violacea* and Fig. 18 for *Operculina Turpethum*. Similar stages have been observed also in the other investigated species belonging to the genus *Ipomea*. The chalazal megaspore functions in every case and develops into the 8-nucleate embryo-sac according to the normal type (Figs. 9–10, 11–13 and 18–20). One or two prominent vacuoles can be discerned in the cytoplasm of the functional chalazal megaspore even before the first division of the nucleus (Figs. 8 and 18). In the 2-nucleate stage of the embryo-sac they are replaced by a prominent central vacuole (Figs. 9 and 19). The enlargement of the embryo-sac at first takes place at the expense of the surrounding nucellus cells. In *Jacquemontia*, most of the nucellus except the outermost layer is absorbed by the 2-nucleate stage. The integument at this stage is 12–15 cells thick. During the course of further development the inner layers of the integument are also absorbed. The 4-nucleate stage of the embryo-sac calls for no remarks. During the mitotic divisions preceding the 8-nucleate stage, of the two spindles at each end, one is placed parallel, the other at right angles to the long axis of the embryo-sac (Fig. 11).

The mature embryo-sac is long and narrow, its length being generally about six times the width (Fig. 16). In *Ipomea pulchella*, it is even longer, and the micropylar end is narrower than the chalazal (Fig. 13). This species is characterised by a very much smaller nucellus and comparatively thicker integument than the rest.

The synergids of *Operculina Turpethum* are nearly as long as broad (Fig. 20) ; of other species they are generally about three times as long as broad (Figs. 10, 13 and 14). The apex of the synergids except in *Operculina* is usually drawn out. Hooks of a small size have been observed on the synergids of *Jacquemontia violacea* (Fig. 10) and *Ipomea pulchella*.

Definite antipodal cells are always formed (Figs. 10 and 12). They are organized generally even before the cells of the egg-apparatus (Fig. 12), but are quite ephemeral and begin to degenerate as soon as they are formed.

The two polar nuclei travel towards the micropylar end of the embryo-sac and stay near the egg-apparatus (Figs. 13 and 20). A fusion of the polar nuclei has not been observed even in those embryo-sacs, where the egg-apparatus appears to be fully ripe. Their fusion,

as pointed out by Raghava Rao (1940), is perhaps delayed until the second male nucleus approaches them.

Macpherson (1921) stated that the cells of the nucellus are rich in starch. Dahlgren (1927) pointed out that these cells may really belong to the integument. The writer's observations as regards the distribution of starch agree with those of Dahlgren. Starch is present in the cells of the integument, but not in the nucellus.

An abnormal embryo-sac has been observed in *Ipomea Horsfalliæ* (Fig. 15). It shows six nuclei at the chalazal end and only two at the micropylar end. Probably one of the micropylar nuclei at the 4-nucleate stage of the embryo-sac was here pushed towards the chalazal end.

SUMMARY

Development of the embryo-sac has been studied in six species of Convolvulaceæ belonging to three genera, namely, *Jacquemontia violacea*, *Ipomea pulchella*, *I. Horsfalliæ*, *I. obscura*, *I. sepiaria* and *Operculina Turpethum*. Two or three primary archesporial cells are occasionally present in *Jacquemontia*. Otherwise there is always only one hypodermal archesporial cell, which differentiates much before the origin of the integument. Parietal tissue is formed in all the species, the archesporial cell cutting off a primary wall cell in every case. The development of the embryo-sac corresponds to the normal type. The antipodals are short-lived. The fusion of the polar nuclei is long delayed.

ACKNOWLEDGMENTS

This investigation was undertaken at the suggestion of Dr. A. C. Joshi of Benares Hindu University, to whom I am greatly indebted for help and advice. I am also thankful to Dr. N. N. Murty for the interest he has shown in the progress of my work and providing the necessary facilities.

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REVIEW

Plant Viruses and Virus Diseases. By F. C. Bawden. Second entirely revised edition; Vol. XIII, 1943, Waltham Mass., U.S.A. Messrs. Chronica Botanica, Calcutta. Messrs. Macmillan & Co., Ltd. Pp. 294. 48 illustrations. Buckram, $ 4·75.

THE publication of this second edition of a quick-selling and important book on a rapidly changing subject is a very welcome enterprise on the part of the editor of the New Series of Plant Science Books, Dr. Frans Verdoorn. The loss of the type of the first edition during the invasion of the Netherlands in 1940, although to be deplored, has resulted in a complete revision of the first edition so as to include all the dynamic changes that have taken place in our 1939-conception of plant viruses. Frequent attempts to bring our knowledge up to date has to be made in this fascinating group of ultra-microscopic plant pathogens which is now the domain of the Pathologist cum Chemist. The opinion of the author that the number of stubborn orthodox biologists who still regard these specific plant virus nucleo-proteins as something other than the viruses themselves has dwindled down, is a tribute to the overwhelming mass of positive evidence that has accrued in the past ten years.

Although this edition has been completely revised, with a number of chapters rewritten, the general arrangement of the subject-matter remains essentially the same as in its predecessor. The chapter headings are: (1) Introductory Survey; (2) and (3) Symptomatology (External and Internal); (4) Transmission; (5) Relationships between viruses and their insect vectors; (6) Virus strains, mutations, and acquired immunity; (7) Serological reactions of plant viruses; (8) Methods of purification; (9) Properties of purified virus preparations; (10) Optical properties of purified virus preparations; (11) Inactivation of viruses; (12) The sizes of virus particles; (13) Physiology of virus diseased plants; (14) The classification of viruses; (15) The control of virus diseases; (16) Discussion on the origin and multiplication of viruses. Bibliographies which are fairly exhaustive and commensurate with the subject-matter dealt with terminate chapters. There are 48 illustrations in all, thus showing an increase of eleven over the first edition.

From the economic point of view the revised chapter on " relations between viruses and their insect vectors," provides a very stimulating reading and in addition permits of visualizing the enormous and complicated problem that this mode of transmission offers in the field. Reverting to the academic problems of plant viruses Mr. Bawden has presented data in a very forceful and lucid way which might be called exact but not exacting. To the academically minded person, therefore, Chapters 7 to 12 will appeal as most convincing evidence of the chemical nature of these plant virus particles. One cannot, however, refrain from remarking that more constructive suggestions on the improvement of virus nomenclature has not found a place in this edition as well.

This, of course, is due to the absorbing interest that a virus pathologist is apt to take on the multifarious aspects of this science—chemical, entomological, serological, etc., that he often finds himself too engrossed to emerge out and tackle the nonetheless intricate and thought-provoking job of introducing the latinized binomial system of nomenclature. From the academic degree point of view in this country it is difficult to introduce virus pathology along with Mycology for the degree courses until the virus nomenclature is put *on a par* with its sister pathological subjects. Nevertheless, Mr. Bawden's book should find a place in the science libraries of every college, for it affords an excellent reading, to the student of general Botany, of a hitherto little known branch of Plant Pathology. To the more advanced Plant Pathologist and Virus Physiologist this neat volume is indispensable, for, it critically sums up all the latest knowledge of *in vitro* and *in vivo* reactions of plant viruses.

T. S. SADASIVAN.

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